VOLUME 7 November 1958 NUMBER 6

THE AMERICAN JOURNAL OF Tropical Medicine and Hygiene

Combining The American Journal of Tropical Medicine and The Journal of the National Malaria Society

OFFICIAL ORGAN OF
THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

PUBLISHED BIMONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, MARYLAND, U.S.A.

© Copyright 1958, By The American Society of Tropical Medicine and Hygiene
Made in U.S.A.

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

Editor-in-Chief, MARTIN FROBISHER
374 Ruckman Road
Closter, New Jersey

Editor, Tropical Medicine and Hygiene News
LOUIS J. OLIVIER, National Institutes of Health
Bethesda 14, Maryland

Editorial Board

G. ROBERT COATNEY, *Chairman*
PAUL C. BEAVER
WILLIAM McD. HAMMON
JOHN M. HENDERSON
HARRY MOST
LLOYD E. ROZEBOOM
ALBERT B. SABIN
LEON H. SCHMIDT

INFORMATION FOR SUBSCRIBERS

THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE is issued bimonthly, appearing in January, March, May, July, September, and November. One volume is issued per year.

Correspondence concerning business matters, subscriptions and orders for reprints, should be addressed to The Williams & Wilkins Company, 428 East Preston Street, Baltimore 2, Maryland, U. S. A.

Manuscripts and Books for Review should be sent to Dr. Martin Frobisher, 374 Ruckman Road, Closter, New Jersey.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received, the pro rata value of such numbers will be refunded to the subscriber.

Subscriptions should be renewed promptly—To avoid a break in your series, subscriptions should be renewed promptly. The publishers cannot guarantee to supply back issues on belated renewals.

Change of address: Publisher must be notified 60 days in advance. Journals undeliverable because of incorrect address will be destroyed. Duplicates can be obtained (if available) from the publisher at the regular price of single issues.

OFFICERS OF THE

American Society of Tropical Medicine and Hygiene

President
DONALD L. AUGUSTINE

President-Elect
LEWIS W. HACKETT

Vice-President
PAUL C. BEAVER

Secretary-Treasurer
ROLLA B. HILL
3575 St. Gaudens Road
Miami 33, Florida

Councilors:	*Term Expires*
MARSHALL C. BALFOUR	1958
THOMAS H. WELLER	1958
LESLIE A. STAUBER	1958
WILLIAM C. REEVES	1959
JOHN M. HENDERSON	1960
J. ALLEN SCOTT	1960
PAUL F. RUSSELL	1961
JOSEPH E. SMADEL	1961

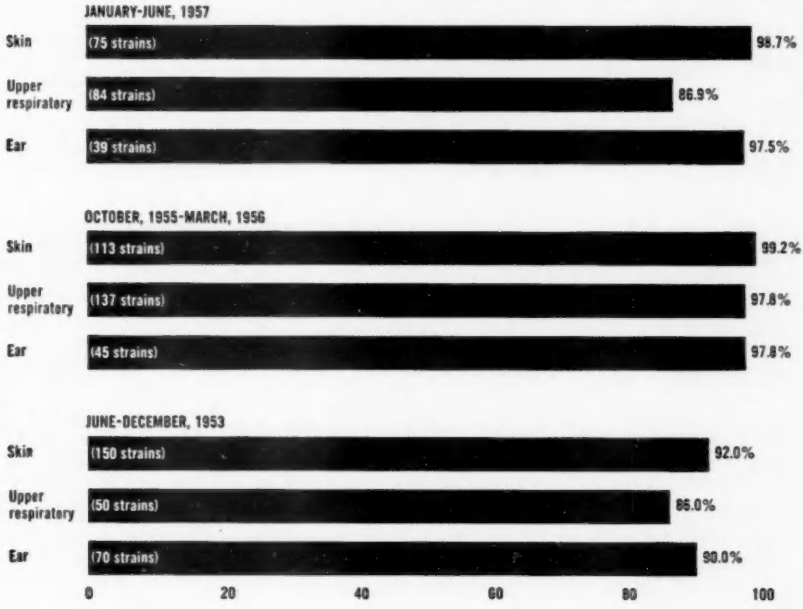
Individuals interested in any phase of tropical medicine and hygiene, including all arthropod-borne diseases, medical care and human welfare are eligible for membership. Dues are $8 a year and include subscription to the American Journal of Tropical Medicine and Hygiene. An application blank is bound into this issue of the Journal for the use of interested individuals. Before being submitted to the Secretary, each application must be signed by two active members of the Society and a check for $8 (U.S. banks preferred) must accompany the application.

Published bimonthly by The Williams and Wilkins Co., 428 East Preston Street, Baltimore 2, Md., U.S.A.
Subscription price $10.00, single issue $2.00
Second class mail privileges authorized at Baltimore, Md.

HIGHLY EFFECTIVE AGAINST STAPHYLOCOCCI ...YEAR AFTER YEAR

CHLOROMYCETIN®

IN VITRO SENSITIVITY OF STAPHYLOCOCCI FROM THREE FOCI OF INFECTION TO CHLOROMYCETIN FROM 1953 TO 1957*



*Adapted from Royer, A., in Welch, H., & Martí-Ibañez, F: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, p. 783.

CHLOROMYCETIN (chloramphenicol, Parke-Davis) is available in a variety of forms, including Kapseals® of 250 mg., bottles of 16 and 100. CHLOROMYCETIN is a potent therapeutic agent and, because certain blood dyscrasias have been associated with its administration, it should not be used indiscriminately or for minor infections. Furthermore, as with certain other drugs, adequate blood studies should be made when the patient requires prolonged or intermittent therapy.

PARKE, DAVIS & COMPANY · DETROIT 32, MICHIGAN

46858

Hastening the decline of malaria

'DARAPRIM'® brand

Pyrimethamine

"... the most efficient suppressive agent known, ..."

"... of extraordinary effectiveness against erythrocytic parasites of all the malarias."

"... probably has some action against the primary exo-erythrocytic forms of all the malarias, ..."

"... is active against the late tissue forms of *Plasmodium vivax* ..."

"... exhibits a phenomenal effect on gametocytes. ... the parasites are not able to develop beyond the early oöcyst stage, thereby blocking the chain of transmission."

Coatney, G. R. : Industry and Tropical Health, Volume II. 1955.

For the treatment of an acute attack 'Daraprim' should be supplemented with a fast-acting schizontocide.

safe • *economical* • *tasteless*

Full information will be sent on request.

Tablet of 25 mg.
Supplied in boxes of 30 and bottles of 1,000.

BURROUGHS WELLCOME & CO. (U.S.A.) INC., Tuckahoe, New York

THE AMERICAN JOURNAL OF
TROPICAL MEDICINE AND HYGIENE

Vol. 7 *November 1958* *No. 6*

CONTENTS

Isolation of Bunyamwera virus from a naturally infected human being and further isolations from *Aedes (Banksinella) circumluteolus* Theo. R. H. Kokernot, K. C. Smithburn, B. de Meillon and H. E. Paterson 579

The serological reactions in yellow fever. Max Theiler and Jordi Casals 585

A new monophasic medium for the cultivation of *Entamoeba histolytica*. Ralph E. Dolkart and Bernard Halpern 595

The first case of sparganosis in Colombia. David Botero and Jose J. Gomez 597

The use of dithiazanine in the treatment of helminthiasis in Mexican farm laborers. E. D. Wagner, F. R. Lemon and H. S. Burnett 600

The isolation and identification of *Trypanosoma cruzi* from raccoons in Maryland. Bryce C. Walton, Preston M. Bauman, Louis S. Diamond and Carlton M. Herman 603

Studies on the use of cortisone and ACTH in trichinosis. Richard A. Lord 611

Development of resistance to molluscicides in *Oncomelania nosophora*. Bryce C. Walton, M. M. Winn and J. E. Williams 618

Biochemical investigations on DDT-resistance in the human body louse, *Pediculus humanus humanus*. Albert S. Perry and Annette J. Buckner 620

An infestation of a human habitation by *Dermanyssus gallinae* (Degeer, 1778) (Acarina: Dermanyssidae) in New York City resulting in sanguisugent attacks upon the occupants. Roger W. Williams 627

Intestinal protozoa and helminths in the peoples of Egypt living in different type localities. R. E. Kuntz, D. K. Lawless, H. R. Langbehn and G. M. Malakatis 630

Biological notes on *Marisa cornuarietis*, a predator of *Australorbis glabratus*, the snail intermediate host of schistosomiasis in Puerto Rico. Frederick F. Ferguson and Juan R. Palmer 640

A skin-test survey for histoplasmosis and coccidioidomycosis in the Middle East. Kamal Abou Daoud and Calvin W. Schwabe 643

Experimental amebiasis—a new approach. R. N. Chaudhuri, T. K. Saha and N. Roy 644

Incidence of leptospirosis in wild mammals from southwestern Georgia, with a report of new hosts for six serotypes of leptospires. S. McKeever, G. W. Gorman, J. F. Chapman, M. M. Galton and D. K. Powers 646

Erythromycin in amebic liver abscess. A. J. Wilmot, S. J. Powell and R. Elsdon-Dew 656

PEAK PERFORMANCE AGAINST INSECT PESTS

TIFA

TODD INSECTICIDAL FOG APPLICATOR

OVER 13 YEARS Successful World-Wide Operation in Public Health Programs

FACTORY MAINTENANCE WINTERTIME REPAIRS · OVERHAULS!

Factory mechanics check, service or recondition your TIFA machines. Get ready for next season ... *let experts* protect your investment! For details, contact either Brooklyn or Houston plant.

Peak performance against all insects pests ... on the dump, over pools, in suburbs, play areas, everywhere insects swarm. Best by test for over 13 years, TIFA sets the records for lowest operating and maintenance costs in over 3000 communities throughout the world. True clean fog with highest efficiency, using newest or older type insecticides (solution, emulsion, suspension) ... one man operation.

Carrying Underwriters' Listing Seal No. MH 4162

Write for illustrated folder and official comments.

TODD PRODUCTS DIVISION SHIPYARDS CORPORATION

PRODUCTS DIVISION
SALES AND SERVICE DEPARTMENTS
Columbia & Halleck Streets, Brooklyn 31, N. Y.

PLANT, SALES AND SERVICE
Houston, Texas

THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

APPLICATION FOR MEMBERSHIP

To the Membership Committee:

The following individual interested in tropical medicine and hygiene is proposed for active membership.

(TYPE OR PRINT)

Name ..... (*Last*) ..... (*First*) ..... (*Middle*)

Mailing Address: Street ..... City .....

State ..... Country .....

Date of Birth ..... Place .....

Present Position .....

Special Field of Interest .....

Education: Institution (Graduate and Postgraduate) Degree Date

.....

.....

.....

Attach to the application a list of professional positions held, membership in scientific societies, and a list of major publications.

* Proposed by:

(1) ....

(2) ..... Signature of Applicant

* Two active members must sign this application.

Return this application to Dr. Rolla B. Hill, Secretary-Treasurer, 3575 St. Gaudens Road, Miami 33, Florida

(Check for $8.00 (U. S. banks requested) must accompany this application.)

notes from a
MICROSCOPIST'S
NOTEBOOK

NATIONAL® PYRONIN Y
CERTIFIED

National was one of the first to produce Pyronin Y in the United States. This xanthene dye, a relatively recent addition to our line, finds its principal use in Scudder's combined Gram-Pappenheim stain for gonorrheal infection. As made by National, Pyronin Y meets every requirement of the Biological Stain Commission.

With assured domestic production of National PYRONIN Y, microscopists are no longer dependent upon the varying availability and quality of foreign material.

In ordering from your supply house, please specify

#710 National PYRONIN Y, Certified

1 gm. $1.20 5 gms. $5.50 10 gms. $10.00 25 gms. $22.50
plus 20¢ certification label charge

NATIONAL
BIOLOGICAL STAINS and INDICATORS

Allied Chemical

NATIONAL ANILINE DIVISION
40 RECTOR STREET, NEW YORK 6, N. Y.

A complete manual of over 50 different pathogenic leptospires . . .

Alston & Broom: LEPTOSPIROSIS IN MAN AND ANIMALS

"Our aim in this book has been to tell what is known about leptospires and the diseases which they cause in man and animals, and to show where new knowledge is at present being gained and may be hoped for in the future. Recently, in the U.S.A., in Malaya, in Israel and in parts of Europe, the disease has been found with increasing frequency, and often caused by serotypes which had not previously been known to be present in those regions.

"It is a matter for concern that, though in human beings the majority of cases are of the milder forms, the disease may require several weeks of convalescence. Also, it has been recognized—only during recent years—that endemic leptospirosis among farm animals may cause serious financial losses in some countries."—*From the Preface*

CONTENTS—MORPHOLOGY, CLASSIFICATION, PHYSIOLOGY AND DISTRIBUTION OF LEPTOSPIRES: History and general survey • Morphology and classification of leptospires • Physiology of leptospires • Reservoir hosts and distribution of leptospires • LEPTOSPIROSIS IN MAN: Epidemiology—Route and means of infection—Incidence with regard to sex, age, occupation, season and year. Morbid anatomy and histology: Clinical pathology. Clinical aspects of severe leptospirosis as exemplified by leptospirosis icterohemorrhagica (Weil's disease). Epidemiological and clinical aspects of milder leptospirosis as exemplified by leptospirosis canicolaris (canicola fever). Epidemiology, pathology and clinical aspects of leptospirosis due to other serotypes. Clinical laboratory diagnosis. Treatment. General prevention and personal prophylaxis. Legal aspects in England and Wales of the contraction of leptospiral diseases. LEPTOSPIROSIS IN ANIMALS: Pigs, cattle, sheep, goats, horses, dogs, foxes, jackals, cats, bats, primates. Treatment and control of leptospirosis in domestic animals. LEPTOSPIROSIS IN REGIONS MOST AFFECTED: Regional occurrence of leptospirosis in the countries most affected. Culture media and laboratory techniques. Bibliography.

By J. M. ALSTON, M.D., F.R.C.P. Edin., Whittington Hospital, London; and J. C. BROOM, O.B.E., M.D., The Wellcome Laboratories of Tropical Medicine, London

380 pp., 36 figs. • $8.50

Publishers of Medical and Scientific Books and Periodicals

SANS TACHE

WILLIAMS & WILKINS COMPANY

BALTIMORE 2, MD.

SHOP BY MAIL

THE WILLIAMS AND WILKINS COMPANY
Baltimore 2, Md.

Please send the following on approval:

Name _____
(Please print)
Address _____
City _____ Zone _____ State _____

☐ Payment enclosed ☐ Bill me.

Shopping by mail is an easy, time-saving way to select books for your personal library.

SMITH SPRAYERS

"For 70 Years Choice for Quality the World Over"

For Mosquito & Malaria Control

For Residual House Spraying or Larviciding Operations. Herculoy Silicon Bronze Sprayers are available in 4 gallon and smaller sizes and Armco Zincgrip steel sprayers are also available in smaller sizes.

SEND FOR CATALOG

On our complete line of hand, continuous, compressed air, knapsack, bucket, wheelbarrow and barrel sprayers. Also hand and crank powder insecticide dusters. We are the originators of sprayers and our products are of outstanding quality in engineering, modern design and performance.

SMITH E-Z KNAPSACK SPRAYER

Maintains continuous high pressure, with only easy slow pumping. Pump entirely of brass to stand long hard usage. Tank solid copper or Armco zinc-grip steel. Equipped with best grade, high-pressure hose, 2-foot brass extension pipe with adjustable brass or fixed type nozzle. New shut-off control may be locked open for continuous spray. Capacity 4 to 5 gallons. Has form-fitting ventilated tank, keeping the back dry and comfortable. Carrying handle; and dasher type agitator which keeps liquid thoroughly mixed. Brass liquid strainer.

One in case. Shipping weight—20 lbs.

SMITH D.D.T. SPRAYER

The SMITH DDT Sprayer consists of a 4 gallon heavy welded galvanized corrugated steel tank, tested for hard service; equipped with brass air pump and brass air-check valve; adjustable carrying strap with snap ends, air-pressure gauge, 7-foot length of oil resisting hose; automatic shut-off control; 2-foot extension rod and all brass precision-built adjustable or fixed type nozzles. A custom built, superior sprayer. Heavy duty industrial use.

One in case. Shipping weight—13 lbs.

SMITH No. 16D DUST KING (quart-size) DUSTER

Unexcelled in performance. Has floating plunger; does not wear out; very easy to operate. Superior in design and construction. Approved by Experiment and Extension Services.

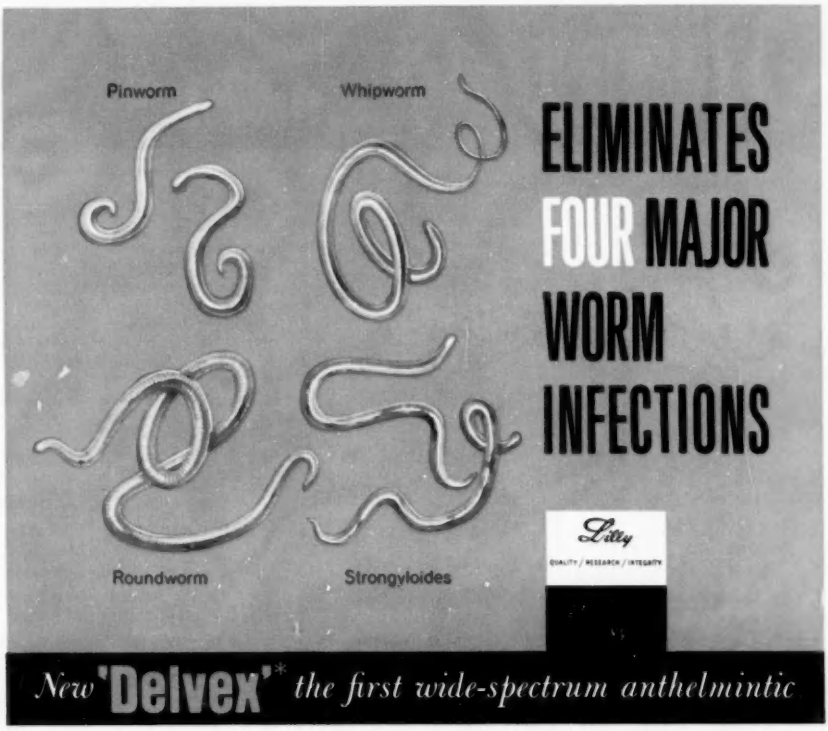
SMITH No. 44 GIANT DUSTER

The Finest Made—Universally Approved

Unexcelled for mosquito and malaria control—when dusting is being done. Duster is precision built, well balanced, very light to carry and easy to operate. Superior in quality and performance.

One in case. Shipping weight—12 lbs.

D. B. SMITH & COMPANY, 432 MAIN ST., UTICA 2, NEW YORK, U. S. A.
ESTABLISHED 1888



Clinical studies[1] *show:*

- 'Delvex' is effective orally, usually within five days, against four of the five most common worm infections:

Pinworm Whipworm

Roundworm Strongyloidiasis

- It also inhibits, and sometimes eliminates, hookworm infection.
- It is fully effective in both single and multiple infections and in both heavy and light infections.
- It eliminates pinworm infection in 100 percent of patients.
- It is the first effective and practicable agent for the oral treatment of strongyloidiasis and whipworm infection.
- No adjunctive measures are needed with 'Delvex' therapy.

Further information may be obtained from your Lilly representative or by writing to our Medical Department.

*'Delvex' (Dithiazanine Iodide, Lilly)
1. Swartzwelder, J. C., et al.: J. A. M. A., 165:2063, 1957.

ELI LILLY AND COMPANY • INDIANAPOLIS 6, INDIANA, U.S.A.

860779

ISOLATION OF BUNYAMWERA VIRUS FROM A NATURALLY INFECTED HUMAN BEING AND FURTHER ISOLATIONS FROM *AEDES* (*BANKSINELLA*) *CIRCUMLUTEOLUS* THEO.[1]

R. H. KOKERNOT,[2] K. C. SMITHBURN, B. DE MEILLON, AND H. E. PATERSON

The Arthropod-Borne Virus Research Unit, South African Institute for Medical Research, Johannesburg

Bunyamwera virus was first isolated by Smithburn *et al.*, (1946) from *Aedes* mosquitoes caught in the Semliki Forest of Uganda. A second strain was isolated from naturally infected mosquitoes (*Aedes* (*Banksinella*) *circumluteolus*) caught in Tongaland, Union of South Africa in 1955 (Kokernot, Heymann *et al.*, 1957). In both these regions neutralizing antibodies against this virus have been found in sera of human beings. This report describes the first isolation of Bunyamwera virus from the blood of a naturally infected human being. The donor was a youth who at the time of his illness was engaged in capturing mosquitoes from which the virus was also isolated.

Field work in Tongaland was initiated in 1954 with the collection of sera from indigenous residents for antiviral immunity tests. This serological survey (Kokernot *et al.*, 1956) yielded a high percentage of sera protective against certain of the arthropod-borne viruses, particularly Bunyamwera and Bwamba fever. Following this, in April–May, 1955, an intensive investigation was conducted which resulted in the isolation of 16 viral strains from mosquitoes and one from man. In the introduction to the series of papers dealing with these results Smithburn and de Meillon (1957) described the unique ecological situation of Tongaland that eventually led to the establishment of a permanent field station in that region near Ndumu. The work at this station has been conducted by field teams since 1956 for 10-day to 2-week periods at monthly intervals from January through July, and at less frequent intervals during the rest of the year. One phase of the program has been the collection of mosquitoes, and another the conduct of clinical sessions to seek out cases of fevers of undetermined origin and possible viral etiology. In both instances specimens are collected for possible virus isolation. The present report is concerned with isolations of Bunyamwera virus from such material collected by field teams in two successive periods of activity, namely April 30 to May 9 and June 21 to 28, 1957.

MATERIALS AND METHODS

Previous reports from this laboratory have described the methods utilized in capturing mosquitoes (de Meillon *et al.*, 1957) and processing them for possible virus isolation (Kokernot, Heymann *et al.*, 1957). At the field station mosquitoes are pooled by species, killed by freezing or with cyanide (Kokernot, de Meillon *et al.*, 1957) and then transferred to sterile Wasserman tubes which are plugged with wax-coated corks. The tubes are then stored in a cabinet or thermos flask containing CO_2 ice until they are processed at the laboratory in Johannesburg.

Clinical sessions are conducted at selected sites in the study area by a qualified medical doctor. All individuals presenting themselves at these clinics receive treatment whatever their complaint; temperatures of all are taken and from those who have fever without obvious cause blood specimens are collected. Pertinent clinical data on each case from which a blood specimen is taken are recorded on a 5″ x 8″ card. On the same card fingerprints of the donor are made to assure correct identification if and when a convalescent specimen is obtained. In the case reported in this paper the initial fingerprints and those taken when the convalescent specimen was obtained were found to be identical.[3]

[1] The studies and observations on which this paper is based were financed jointly by the South African Institute for Medical Research, the Poliomyelitis Research Foundation, the South African Council for Scientific and Industrial Research, and The Rockefeller Foundation, and were conducted with the collaboration of the Union Health Department.

[2] Address: South African Institute for Medical Research, P.O. Box 1038, Johannesburg, South Africa.

[3] We acknowledge with thanks the examination and opinion of these prints by a member of the South African Police force, Lt. G. Retief, Officer-in-Charge, Local Finger Print Office, Johannesburg.

The specimens collected at each clinic are allowed to clot and then placed in a thermos flask containing water ice or in a 4°C refrigerator until the clot has retracted. The serum is then removed and stored on CO_2 ice in sterile Lusteroid tubes. On arrival at the central laboratory the sera are allowed to thaw, one or two litters of young mice are inoculated intracerebrally with 0.02 ml each and six adult mice are similarly inoculated, but with 0.03 ml of serum.

Convalescent specimens are obtained from many cases during the subsequent field trip. However, in the case reported here this was impossible because the donor had left the area and could not be found for some time. The second specimen was collected 134 days after the original had been obtained.

The method used in identifying the virus strain from the human being and mosquitoes was similar to that described by Smithburn *et al.*, (1957). Preliminary screening complement-fixation (CF) tests were done with a crude antigen prepared by making a 10 per cent suspension of infected infant mouse brain in chilled borate saline pH 9.0 and centrifuging at 10,000 rpm for 1 hour. The supernate was used as the antigen.

Casals *et al.*, (1951), have described the method utilized in doing CF tests. Hemagglutination-inhibition (HI) tests were done according to methods described by Clarke and Casals in special reports from the laboratories of The Rockefeller Foundation in New York (Clarke and Casals, 1958). For use in the HI tests, Bunyamwera antigen was prepared by sucrose-acetone extraction. Acute and convalescent sera were heat-inactivated in a 60°C water bath for 30 minutes. Use of goose erythrocytes for the test was based on the report by Porterfield (1957) who found them superior to day-old-chick cells. The species of goose used by that investigator was not available, but cells from the common grey goose (referred to as Emden by the supplier) proved satisfactory.

The strain of Bunyamwera virus used in neutralization (N) studies was the original isolate described by Smithburn *et al.*, (1946) in its 44th mouse-brain passage.

RESULTS

Isolation and identification of virus strain from the human being. An African male (S.M.), aged 13 years, resumed employment with the arrival at Ndumu of the field team on April 30, 1957, and assisted in making mosquito collections. He was born and reared in the immediate vicinity where such activity was being conducted and denied ever having travelled outside the area. At noon of May 9th, after having participated in making a morning's catch, he reported sick to one of us (K.C.S.). His only complaint was of a headache which had its onset that morning on awakening. At first it was not incapacitating, but by noon it had become progressively more severe and he sought medical attention. On physical examination he appeared to be a fairly well-developed African male with fine features, in good nutritional status, but showing evidence of acute distress. His oral temperature was 102.8°F. No other positive physical signs were found in examination of ears, throat, ocular movements, lungs, heart, abdomen and superficial and deep tendon reflexes. A venipuncture was made and about 15.0 ml of blood taken. The specimen was designated H 427. Serum was separated late that afternoon and stored on CO_2 ice. The clinical course of illness in this case was not followed because field activities for that particular period were terminated the same day. However, when questioned at the time of obtaining a convalescent specimen 134 days later, he remarked that in addition to the headache he had developed a stiff neck. He denied any residual subjective symptoms and appeared to be normal. He was then employed as a domestic servant and his employer had not noticed any evidence of incapacitation.

In the single litter of seven newborn mice inoculated at the central laboratory in Johannesburg on May 11th, two were dead and five were sick on the second postinoculation day. Brains of the latter group were harvested for passage, pathological examination and storage. Passage was successful and a serially transmissible agent was established. The infective agent was successfully reisolated from serum that was again inoculated into a litter on May 13th. Suspensions of brains of sick mice receiving the inoculum failed to produce evidence of bacterial growth when cultured on nutrient broth or blood agar.

The filterability of the infective agent was readily demonstrated by its passage through an asbestos filter pad (Ford's Sterimat S.B. grade). The first two mouse-brain passages were not pathogenic for young adult mice when inoculated intracerebrally. However, the virus was readily adaptable. A fifth-passage infected adult mouse brain gave a titer of 346,700 mouse-intracerebral

LD_{50}/0.03 gm. After additional passages, potent stocks of lyophilized virus were prepared and referred to as H 427 strain corresponding to the field number of the blood specimen from which it was isolated.

In order to obtain homologous immune serum a nonimmune *Cercopithecus aethiops pygerythrus* F. Cuvier monkey was inoculated intracerebrally with a fresh infant-mouse-brain preparation that titered in excess of 800,000 infant-mouse-intracerebral LD_{50}/0.02 gm. The monkey showed no clinical signs, but had a detectable viremia on the first postinoculation day. Subsequent tests confirmed the identity of the agent as being the same as that inoculated.

In an initial CF screening test, undiluted H 427 virus antigen was tested against undiluted mouse-hyperimmune sera for Bunyamwera virus and four Tongaland virus isolates (AR 136,[4] AR 344,[4] H 336[4] and H 177). The results of this test indicated the fixation of complement by H 427 antigen and Bunyamwera antiserum. There was no such reaction with the other antisera and all of the serum controls were negative.

A quantitative CF test with this antigen was then done utilizing, in addition to antisera prepared from the Uganda strain of Bunyamwera virus, the strain isolated in Tongaland designated AR 11 (Kokernot, Heymann *et al.*, 1957). The results gave further evidence of the relationship of H 427 viral strain to Bunyamwera virus (Table 1).

Intracerebral neutralization tests in adult mice with H 427 and Bunyamwera viruses were done against the acute and convalescent-phase sera of the donor and pre- and postinoculation Bunyamwera monkey sera. Sera from both sources were heat-inactivated for 1 hour at 56°C prior to testing. The acquisition of immunity against the homologous agent and Bunyamwera virus during convalescence of the donor indicated the origin of the virus from the individual and its relationship to Bunyamwera. The results shown in Table 2 indicate furthermore that there is a significant reciprocal cross-relationship of the two viral strains.

Results of in vitro serologic tests showed that the donor had a significant increase in titer of Bunyamwera HI antibodies. The acute specimen failed to inhibit agglutination at a dilution of

[4] Strains of virus isolated in this laboratory not yet firmly identified.

TABLE 1

Results of complement-fixation test with H 427 virus antigen and hyperimmune sera for two strains of Bunyamwera virus

Antigen	Mouse hyperimmune serum*											
	Bunyamwera, 1 in						AR 11, 1 in					
	8	16	32	64	128	256	8	16	32	64	128	256
H 427, 1 in												
4	4	4	4	4	4	4	4	4	4	4	4	4
8	4	4	4	4	4	3	4	4	4	4	4	4
16	4	4	4	4	4	3	4	4	4	4	4	4
32	4	4	4	4	4	4	4	4	4	4	4	4
64	3	4	4	4	4	2	2	2	3	2	3	1
128	2	2	2	2	2	1	0	0	0	0	0	0

* Serum control negative.

TABLE 2

Results of intracerebral adult mouse neutralization tests with H 427 virus strain showing origin from a human being and relationship to Bunyamwera virus

Serum*	Virus			
	H 427		Bunyamwera	
	Titer of virus, log	Logs virus neutralized	Titer of virus, log	Logs virus neutralized
Donor, acute	6.9		6.3	
Donor, convalescent	3.4	3.5	2.7	3.6
M 230 preinoculation	6.5		6.8	
M 230 post-Bunyamwera	4.0	2.5	3.5	3.3

* All sera tested after heat inactivation for 1 hour at 56°C.

TABLE 3

Results of three types of tests with acute and convalescent sera from donor of H 427 strain against Bunyamwera virus or antigens therefrom

Serum	Type of test		
	Neutralization	Hemagglutination-inhibition	Complement-fixation
	Titer	Serum dilution	
Acute (5.9.57)	$10^{-6.3}$	<1:10	<1:8
Convalescent (9.20.57)	$10^{-2.7}$	1:40	1:8

TABLE 4

*Results of cross-neutralization tests with Bunyamwera, H 427, AR 800 and AR 802 virus strains and their respective pre- and postinoculation sera**

Serum	Virus strain, logs neutralized			
	Bunyamwera	H 427	AR 800	AR 802
Bunyamwera..	3.3†	2.5†	3.3	3.1
H 427.....	3.6*†	3.5*†	4.1	2.6
AR 800.....	3.9	3.6	4.3	2.6
AR 802.....	4.3	3.2	4.6	2.9

* Paired sera from the human being from which H 427 was isolated used in tests with that virus and Bunyamwera.

† Sera heat-inactivated one hour at 56°C prior to testing.

1:10, but the convalescent specimen inhibited this reaction at a serum dilution of 1:40. Bunyamwera CF antibodies were demonstrable only in a 1:8 dilution of the convalescent specimen, but were not detectable in the same dilution of acute-phase serum. These results and those of the N tests are summarized in Table 3.

Isolation and identification of viral strains from mosquitoes. Two of the three strains were isolated from mosquitoes caught during the period of field activity April 20 to May 9, 1957; the third was isolated from mosquitoes caught in June. All three agents came from pools of *Aedes* (*Banksinella*) *circumluteolus*. The pool designated AR 800 consisted of 431 mosquitoes of this species caught May 6th and that designated AR 802 consisted of 408 caught May 7th. In the latter pool 49 of the mosquitoes were collected from a tree platform 46 feet above the ground level where the remainder were collected. On May 13th the pools were suspended separately in 10.0-ml portions of bovine plasma albumin and after centrifugation and addition of antibiotics the supernates were each inoculated intracerebrally into a litter of newborn mice and a group of six adults. In each case the original inoculation and a repeat inoculation done 2 days later resulted in sickness or death of infant mice on the second postinoculation day. The infective agents were successfully passed through an asbestos filter and subsequently referred to as AR 800 and AR 802 virus strains.

On first passage neither of the strains caused sickness in adult mice, but second passage AR 802 produced 100 per cent mortality in the six adult mice inoculated intracerebrally with a 10 per cent infected infant-mouse-brain suspension. A fourth-passage adult-mouse brain contained 20,000,000 intracerebral-adult-mouse LD_{50}/0.03 gm. AR 800 virus did not adapt to the older mice as readily. At the fourth-passage level a 10 per cent suspension of infected infant-mouse brain caused only 50 per cent mortality in the six adults inoculated. However, by the sixth passage it regularly caused death of adult mice and its titer was comparable to that of the AR 802 strain.

Guinea pigs were inoculated intracerebrally (after first obtaining preinoculation blood specimens) with each virus strain in order to determine their identity and relationships. The results of cross-neutralization tests with AR 800, AR 802, H 427 and Bunyamwera viruses against their respective pre- and postinoculation guinea-pig or monkey sera are shown in Table 4. These results also incorporate those presented in Table 2 in which H 427 and Bunyamwera viruses were tested against the paired sera from the human being. The results of these tests indicate the relationship of the virus strain isolated from the human being with the two strains derived from mosquitoes. Furthermore, the results show that all three are identical with, or closely related to, Bunyamwera virus.

The third virus strain, designated AR 825, was isolated from a pool of 150 *Aedes* (*Banksinella*) *circumluteolus* mosquitoes collected June 27th, which was during the subsequent field trip. The pool was processed and inoculated on July 1st in the same manner as that described above for the other two strains of virus derived from mosquitoes. Of the 12 newborn mice (two litters) inoculated, all were either dead or sick on the third postinoculation day. The group of six adult mice similarly inoculated remained well for the 21-day observation period. However, the agent was readily adaptable to the older mouse, as evidenced by 100 per cent mortality in the group inoculated with first-passage infected infant-mouse brain.

The infective agent was reisolated from the mosquito suspension after storage for 3 days at −20°C.

Utilizing AR 825-infected mouse brains and pre- and postinoculation Bunyamwera monkey serum, an intraperitoneal N test was done in newborn mice. The virus titer in the preinoculation serum was $10^{-7.85}$, while in the postinocula-

tion serum it was $10^{-2.62}$. An intracerebral adult-mouse N test with AR 825 virus was done against pre- and postinoculation AR 800 guinea-pig serum. In the preinoculation serum the titer was 10^{-7} and in the postinoculation serum, $10^{-3.87}$. In each test AR 825 virus was neutralized to a significant degree by sera for two strains of Bunyamwera virus. It is thus clear that AR 825 strain is related to, or identical with, Bunyamwera virus.

DISCUSSION

In the paper reporting the original isolation of Bunyamwera virus, serological evidence was presented that this virus attacks man. Subsequently Southam and Moore (1951) described severe encephalitis in a human being inoculated intravenously with the virus. The results presented in the present communication furnish additional evidence that Bunyamwera virus in nature is a causative agent of disease in human beings. The isolation and successful reisolation of Bunyamwera virus from the blood during the acute illness and the demonstration of development of significant neutralizing antibodies to the homologous agent following the attack, clearly indicate infection with this virus.

The immunological pattern in the case reported in this paper is similar to that noted in sera of individuals convalescent from infection with other of the arthropod-borne viral agents. This observation has been that both neutralizing and HI antibodies are more persistent than CF antibodies.

It is highly probable that the mode of infection in this case was by a mosquito vector, probably *Aedes* (*Banksinella*) *circumluteolus*. The person from whom the virus was isolated was engaged in catching mosquitoes, and from two lots of this species caught at that time strains of the same agent were derived. In a previous study, de Meillon *et al.*, (1957) cite results of precipitin tests which indicate that this mosquito species occasionally feeds on man. Utilizing laboratory-reared *Aedes* (*Banksinella*) *circumluteolus*, Paterson (to be published) has shown this species to be a capable vector of Bunyamwera virus as indicated by successful mouse-mosquito-mouse transmissions.

From the time when mosquito-catches were made that yielded strains of this virus, and the onset of clinical symptoms, it is possible to postulate the period of incubation. AR 800 strain was isolated from mosquitoes collected May 6th and AR 802 from mosquitoes collected May 7th, which were 3 and 2 days, respectively, before onset of symptoms May 9th. This interval corresponds to the 3-day incubation period in the case of infection induced by inoculation (Southam and Moore, 1951).

The isolation of Bunyamwera virus from blood of a naturally infected human being gives increased significance to the results of serological surveys which have previously been reported. The public health importance of this virus as a causative agent of disease in man should be apparent in regions where protective sera have been found. Thus it should be considered important in such regions as East Africa (Smithburn, 1952) and Borneo (Smithburn, 1954) where protective sera have been found (12/296 and 6/49, respectively). Because of the relatively small sample of the total population represented in these surveys, one might anticipate an even higher incidence of immunity among residents of certain ecological niches. In South Africa, for example, the immunity rate to Bunyamwera virus reported in the serological survey for neutralizing antibodies to that agent in the Union of South Africa was 41/1,365. Of this number, 16 of the protective sera were detected among 35 residents sampled at Simbu Pan in Tongaland. An intensive survey has been made of sera collected from numerous other localities in Tongaland, and from this study (Smithburn *et al.*, in preparation) it appears that there are very definite ecological patterns associated with a high incidence of immunity to Bunyamwera virus.

SUMMARY

1. The isolation of a strain of Bunyamwera virus from the blood of a naturally infected human being is described.

2. Following infection there was a significant increase in titer of both neutralizing and hemagglutination-inhibiting antibodies in tests controlled by the inactivated acute serum from which the virus was isolated.

3. The significance of Bunyamwera virus as a causative agent of disease in human beings is discussed.

4. Three strains of this virus were isolated from *Aedes* (*Banksinella*) *circumluteolus* mosquitoes and two of these were from insects collected during the same time period and in the same locality where the human case acquired infection.

5. Evidence is discussed which would indicate

that this mosquito species is a potential vector of Bunyamwera virus.

REFERENCES

CASALS, J., OLITSKY, P. K., AND ANSLOW, R. O., 1951. A specific complement-fixation test for infection with poliomyelitis virus. *J. Exp. Med.*, **94:** 123–137.

CLARKE, D. H., AND CASALS, J., 1958. Techniques for hemagglutination and hemagglutination inhibition with arthropod-borne viruses. *Am. J. Trop. Med. and Hyg.*, **7:** 561–573.

DE MEILLON, B., PATERSON, H. E., AND MUSPRATT, J., 1957. Studies on arthropod-borne viruses of Tongaland. II. Notes on the more common mosquitoes. *S. Afr. J. Med. Sci.*, **22:** 47–53.

KOKERNOT, R. H., SMITHBURN, K. C., AND WEINBREN, M. P., 1956. Neutralizing antibodies to arthropod-borne viruses in human beings and animals in the Union of South Africa. *J. Immunol.*, **77:** 313–323.

KOKERNOT, R. H., DE MEILLON, B., PATERSON, H. E., HEYMANN, C. S., AND SMITHBURN, K. C., 1957. Middelburg virus. A hitherto unknown agent isolated from *Aedes* mosquitoes during an epizootic in sheep in the eastern Cape Province. *S. Afr. J. Med. Sci.*, **22:** 145–153.

KOKERNOT, R. H., HEYMANN, C. S., MUSPRATT, J., AND WOLSTENHOLME, B., 1957. Studies on arthropod-borne viruses of Tongaland. V. Isolation of Bunyamwera and Rift Valley fever viruses from mosquitoes. *S. Afr. J. Med. Sci.*, **22:** 71–80.

PORTERFIELD, J. S., 1957. Use of goose cells in haemagglutination tests with arthropod-borne viruses. *Nature*, **180:** 1201–1202.

SMITHBURN, K. C., HADDOW, A. J., AND MAHAFFY, A. F., 1946. A neurotropic virus isolated from *Aedes* mosquitoes caught in the Semliki Forest. *Am. J. Trop. Med.*, **26:** 189–208.

SMITHBURN, K. C., 1952. Neutralizing antibodies against certain recently isolated viruses in the sera of human beings residing in East Africa. *J. Immunol.*, **69:** 223–234.

SMITHBURN, K. C., 1954. Neutralizing antibodies against arthropod-borne viruses in the sera of long-time residents of Malaya and Borneo. *Am. J. Hyg.*, **59:** 157–163.

SMITHBURN, K. C., AND DE MEILLON, B., 1957. Studies on arthropod-borne viruses of Tongaland. I. The expedition of April–May 1955. *S. Afr. J. Med. Sci.*, **22:** 41–46.

SMITHBURN, K. C., KOKERNOT, R. H., WEINBREN, M. P., AND DE MEILLON, B., 1957. Studies on arthropod-borne viruses of Tongaland. IX. Isolation of Wesselsbron virus from a naturally infected human being and from *Aedes (Banksinella) circumluteolus* Theo. mosquitoes. *S. Afr. J. Med. Sci.*, **22:** 113–120.

SOUTHAM, C. M., AND MOORE, A. E., 1951. West Nile, Ilheus and Bunyamwera virus infection in man. *Am. J. Trop. Med.*, **31:** 724–741.

THE SEROLOGICAL REACTIONS IN YELLOW FEVER

MAX THEILER AND JORDI CASALS

The Rockefeller Foundation Virus Laboratories, New York

Knowledge of the immunological relationships among the arthropod-borne animal (arbor) viruses has increased considerably in recent years. The systematic study of these relationships has been greatly stimulated by the development of hemagglutinins for a large number of these agents (Sabin and Buescher, 1950; Sabin, 1951; Sweet, Chanock and Sabin, 1953; Casals and Brown, 1954; Clarke and Theiler, 1955). By the use of hemagglutination inhibition (HI) tests, Casals and Brown (1954) showed that yellow fever is closely related to a number of other arbor viruses, among them dengue type 1, dengue type 2, Ilheus, West Nile, St. Louis, Japanese B and Russian spring-summer encephalitis. These immunologically related agents, together with certain others, were named Group B arbor viruses. The marked immunological overlap between members of Group B, as shown not only by HI but also by complement-fixation (CF) and at times by neutralization (N) tests, made it desirable to evaluate the usefulness of these tests in the diagnosis of human infections due to Group B viruses.

The recent occurrence of human cases of yellow fever in Trinidad and near Belem, Brazil, offered an opportunity for a detailed study of the immunological response in this infection. In each case diagnosis of yellow fever was made by isolation of the virus from acute-phase sera and indentification by N tests with known yellow-fever-immune sera. The isolation and identification of the Trinidad strains has been described by Downs (1955). In this paper the Trinidad cases are designated by the case number preceded by the letters Tr. All the virus strains from Brazil were isolated by Dr. O. R. Causey, of the Belem Virus Laboratory, and sent to these laboratories for identification. The Brazilian cases are labeled AP or UR. It should be emphasized that the diagnosis was made by the isolation and identification of virus from each case and not by serological studies of the convalescent-phase sera.

METHODS AND MATERIALS

The techniques for the preparation of antigens and the methods for carrying out CF and HI tests have been described (Casals and Palacios, 1941; Casals and Brown, 1954; Clarke and Casals, 1955). Antigens for either test consisted of acetone-ether extracted brain tissue from newborn mice inoculated with the corresponding virus. In the CF test, 8 units of antigen were used and the sera were tested in serial 2-fold dilutions beginning at 1:4. The titer of a serum is recorded in the tables as the denominator of the dilution of serum that gave 3 plus or better fixation. In the HI tests also, 8 units of antigen were used. Following removal of nonspecific inhibitors by means of bentonite, the sera were tested in serial 2-fold dilutions commencing at 1:10. The titer of a serum is recorded in the tables as the ordinal of the tube in which the highest dilution of serum gave complete inhibition of hemagglutination; for example, 1 equals a titer of 1:10, 2 a titer of 1:20 and so on.

The N test by intracerebral route of inoculation of 0.03 ml into young adult mice was used. The mixtures of serum and virus were prepared so as to contain approximately 100 LD_{50} of virus in 0.03 ml of the mixture and incubated for one hour at 37°C before inoculation. Six mice were inoculated with each serum-virus mixture; in each test a titration of the virus suspension was included. The results of the neutralization tests are recorded in the tables according to the protective ratio (PR), that is, mice surviving of six inoculated, or the average survival time (AST); the latter is often of value in indicating a slight neutralizing action of sera. Neutralization tests were observed for 21, 17, 14 and 10 days, respectively, for dengue type 1, dengue type 2 (Tr 1751), Ilheus and yellow fever. The following virus strains were used: dengue type 1, Hawaii; dengue type 2, Tr 1751; Ilheus, Laemmert and Hughes; St. Louis, Parton; yellow fever (French neurotropic for N tests, and JSS for HI and CF test antigens); West Nile, Egypt 101.

With the exception of West Nile, all the viruses in the various tests were either known to be, or suspected of being, present in Trinidad or the Amazon Valley.

As far as quantities permitted, all the acute and convalescent sera were tested in the HI and

TABLE 1

Serological reactions in primary yellow fever infection

Case No.	Days after onset	Hemagglutination inhibition						Complement fixation					Neut. test YF	
		YF	Ilh.	Den 1	WN	Tr 1751	St. L.	YF	Ilh.	Den 1	St. L.	Tr 1751	PR	AST
Tr 2942	4	0	0	0	0		0	0	0	0	0		1/6	7.0
	36	7	5	2	5	3	5	Tr	0	0	0		1/6	7.0
	103	5	3	0	3		4	16	0	0	0		6/6	*10.0*
	441	0	0	0		0	0	0	0	0	0	0		
Tr 4403	2	1	0	0	0		0	0	0	0	0		0/6	5.0
	50	5	5	2	5		5	16	0	0	0		4/6	9.2
	293	3	1	1		1	2	32	0	0	0	0		
Tr 4754	5	6	5	2	4		4	8	0	0	0		3/6	9.8
	40	6	6	3	5		5	32	0	0	0		5/6	*9.5*
Tr 5558	48	5	5	2	5		5	8	0	0	0		6/6	*10.0*
UR 29	−9*	0	0	0	0			0	0	0	0		0/6	5.2
	29	5	3	0	5			Tr	0	0	0		4/6	9.3
	59	5	2	1		3	3	64	Tr	0	4	0		
UR 46	3	0	0			0	0	0	0	0	0		0/6	5.5
	47	7	4	0	6			16	0	0	0		6/6	*10.0*
	77	3	2	1		3	2	64	0	0	0	0		
UR 47	3	0	0	0		0	0	0	0	0	0		2/6	7.3
	47	7	4	0	6			32	0	0	0		6/6	*10.0*
	77	2	1	0		2	1	128+	0	0	0	0		
UR 57	2	0	0	0		0	0	0	0	0	0	0	0/6	5.2
	50	4	2	1		2	2	64	0		0	0		
	89	3	1	0		1	1	32	0		0	0		
UR 220	2	0	0	0		0	0						0/6	5.0
	40	6	4	4		5	5	128+	32		16	0	6/6	*10.0*
AP 7	3	0	0	0		0	0						0/6	5.3
	18	3	2	0		2	2	8	0		0	0	6/6	*10.0*
AP 22	2	0	0	0		0	0	0	0		0	0	0/6	5.2
	11	2	0	0		0	0	0	0		0	0	4/6	8.7
AP 51	4							0	0	0	0	0	0/6	5.0
	26	2	0			0	1	16	0	0	0	0	5/6	*9.8*
AP 55	3	0	0	0		0	0	0	0		0	0	0/6	5.5
	11	3	0	0		0	0	0	0		0	0	6/6	*10.0*
	24	4	2	2			4	128+	16		4	0	4/6	9.3
AP 67	4	0	0			0	0	0	0	0	0	0	0/6	5.5
	26	5	3			3	5	64	8	0	0	0	4/6	9.2
AP 97	2	0	0			0		0	0	0	0	0	0/6	5.3
	23	4	2			2		Tr	0	0	0	0	2/6	8.5
AP 100	3	0	0			0		0	0	0	0	0	0/6	5.5
	24	4	2			1		32	0	0	0	0	4/6	9.3
AP 53	2	0	0			0	0	0	0	0	0	0	0/6	5.2
	24	2	0			0	2	8	8	4	Tr	Tr	1/6	8.5

* Sera obtained for routine survey purposes before the onset of the yellow fever epidemic.

CF tests with a battery of Group B antigens and in N tests with yellow fever, Ilheus, dengue 1 and Tr 1751 viruses.

RESULTS

Soon after the initiation of these studies it became apparent that the in vitro results could be roughly divided into two groups according to the pattern of the serological reactions. The first group (Table 1) is composed of those cases in which the convalescent sera (with one exception) gave clear-cut, positive CF reactions with yellow fever antigen only. Occasionally (UR 29, UR 220, AP 55, AP 67, AP 53) positive reactions

TABLE 2

Serological reactions in cases of secondary infection with yellow fever

Case No.	Days after onset	Hemagglutination inhibition						Complement fixation					Neut. test YF	
		YF	Ilh.	Den 1	WN	Tr 1751	St. L.	YF	Ilh.	Den 1	St. L.	Tr 1751	PR	AST
Tr 4140	0	3	3	1	2		3	0	0	0	0		0/6	5.3
	109	7	9	7	9		9	16	128	16	128		*6/6*	10.0
Tr 4317	0	7	9	8	9		9	0					0/6	6.0
	22	10+	10+	10+	12		11	16	128+	128+	128+		*6/6*	10.0
Tr 5689	12	7	10+	7	9		9						2/6	7.5
	36	10	10+	9	11		11	128	128+	32	128+		2/6	8.7
Tr 5900	5	5	7	5	7		7	0	16	4	16		0/6	5.8
	35	10+	10+	9	11		11	32	128+	64	128+		*5/6*	9.6
	295	7	9	6		8	9	64	128	8	64	64		
UR 11	−25*	1	0	0	1			0	0	0	0		0/6	5.3
	28	9	10	9	10+	9		32	128+	0	64		*4/5*	9.8
	60	7	9	7		9	9	128+	128+	0	128	16		
UR 19	−16*	0	6	0	4			0					0/6	4.8
	25	9	10+	8	10+			4	128+	4	64		*6/6*	10.0
	58	8	10	8		9	9	128+	128+	8	128+	64		
UR 48	3	0	0	0		0	0	0	0	0	0		1/6	5.5
	47	9	10+	8	10+			32	128+	0	64		*6/6*	10.0
	77	6	9	6		8	8	64	64	4	64	8		
UR 51	2	0	0	0	0			0	0	0	0		0/6	4.7
	35	7	3	5	9			16	16	0	8		*6/6*	10.0
	65	4	5	3		5	5	16	16	0	8	0		
UR 79	1	2	4	0	4			0	4	0	0		0/6	5.0
	31	9	10+	8	10+			8	128+	8	128+		*6/6*	10.0
	84	6	9	6		8	8	16	64	4	64	8		
AP 24	1	0	2	0		1	0	0	0		0	0	0/6	5.2
	10	9	12+	9		11	11	128+	128+		128+	128+	*5/5*	10.0
AP 96	3	0	0	0				0	0	0	0	0	0/6	6.3
	26	6	5	6				64	64	0	32	8	*6/6*	10.0
AP 66	3							0	0	0	0	0		
	25	10+	10+			10+	10+	128+	128+	32	128+	128+	2/6	8.7

* Sera obtained for routine survey purposes before the onset of the yellow fever epidemic.

occurred with other antigens; except for AP 53, however, these heterologous reactions were much weaker than with yellow fever. The same sera all inhibited hemagglutination by yellow fever antigen and ordinarily gave some inhibition with one or more of the other antigens used. Typically, inhibition of the homologous (i.e., yellow fever) antigen occurred earlier in convalescence and at higher dilutions than inhibition of the heterologous antigens. Although in two cases one or more of the heterologous reactions were equal to the homologous one, the heterologous reaction never exceeded the yellow fever effect.

A second group of sera from 12 convalescent individuals exhibited strikingly different patterns in the CF and HI tests (Table 2). The sera fixed complement with at least three, and frequently with all five, of the antigens used. In spite of the fact that the diagnosis of yellow fever was never in question (virus was isolated in all cases), titers obtained with heterologous antigens were frequently higher than those obtained with yellow fever.

The HI test also revealed a broad spectrum of heterologous antibodies. The sera inhibited to some degree all of the six antigens employed, and in 10 of the 12 cases one or more of the heterologous reactions occurred in higher dilutions than did the homologous one.

It seems highly improbable that the differences observed in the reactions of these two groups of sera could have occurred by chance. A more likely eixplanaton is to be found in the hypothesis that the first group of convalescent

TABLE 3

Results of neutralization tests with acute and convalescent sera from primary cases of yellow fever

Case No.	Days after onset sera obtained		Virus strains used in tests											
			Dengue type 1				Dengue Tr 1751				Ilheus			
			Acute		Conval.		Acute		Conval.		Acute		Conval.	
	Acute	Conval.	AST	PR	AST	PR	AST	PR	AST	PR	AST	PR	AST	PR
Tr 2942	4	36	13.7	1/6	14.8	0/6					6.8	0/6	7.3	0/6
		103			13.3	0/6							6.8	0/6
		441											6.3	0/6
Tr 4403	2	50	13.5	0/6	18.5	4/6					7.0	0/6	7.3	0/6
Tr 4754	5	40	12.8	0/6	15.0	1/5					7.7	0/6	7.2	0/6
Tr 5558		48			16.3	1/6							6.0	0/6
UR 29	−9*	29	14.0	0/6	14.5	1/6	9.2	0/6	11.0	0/6	6.2	0/6	6.7	0/6
		59											6.7	0/6
UR 46	3	47			15.8	1/6	8.7	0/6	11.3	0/6	7.0	0/6	6.8	0/6
		77							11.0	0/6			6.8	0/6
UR 47	3	47			17.2	1/6	8.7	0/6	11.2	0/6	6.8	0/6	6.3	0/6
		77			11.5	0/6			9.8	0/6			7.0	0/6
UR 57	2	50	13.0	0/6	12.8	0/6	10.5	0/6	10.2	0/6	6.5	0/6	6.3	0/6
		89			12.3	0/6			9.8	0.6			7.2	0/6
UR 220	2	40			18.3	3/6			11.2	0/6			6.7	0/6
AP 7	3	18			14.0	0/6			9.8	0/6			7.2	0/6
AP 22	2	11			12.7	1/6	9.6	0/6	10.0	0/6			6.7	0/6
AP 51	4	26			12.3	1/6							6.5	0/6
AP 55	3	11	11.7	0/6	11.5	0/6	8.8	0/6	9.3	0/6	6.7	0/6	8.2	0/6
		24			15.2	2/6								
AP 67	4	26	14.5	2/6	11.5	0/6					7.0	0/6	7.0	0/6
AP 97	2	23	14.2	1/6	13.5	2/6					6.5	0/6	6.7	0/6
AP 100	3	24	11.2	0/6	14.3	1/6					6.7	0/6	7.3	0/6
AP 53	2	24			14.0	2/6							6.5	0/6

* Sera obtained for routine survey purposes before the onset of the yellow fever epidemic.

sera came from persons who had not previously suffered from an infection with a Group B virus. The second group would then represent cases in which the current attack of yellow fever occurred against a background of previous Group B infection.

Such an explanation is consistent with experiments performed in this laboratory in which animals were infected first with one and later with another virus of the same group (Casals, 1957). The human sera alone, however, provide strong supporting evidence for the assumption. Table 3 and the last two columns of Table 1 reveal that none of the patients assigned to group I on the basis of the reactions of their convalescent sera possessed protective antibodies against any of the Group B viruses prior to their infection with yellow fever. Table 1 shows furthermore that none of these patients had hemagglutinating or CF antibodies against any Group B antigen prior to infection with yellow fever. The only acute serum giving positive reactions in the HI and CF tests and partial protection in the N test was taken on the fifth day of illness, late enough to allow demonstration of antibodies produced in response to the current infection (Tr 4754).

In sharp contrast to the foregoing results, almost all the acute sera from group II patients gave evidence of previous attacks of Group B infection (Table 4). This evidence varies in degree. Four of the 11 sera tested (Tr 5689, Tr 5900, UR 51 and UR 79) gave clear-cut protection against Ilheus virus. One of these gave complete, and another (Tr 5689) partial, protection against dengue 1 as well. A fifth serum (Tr 4317) also gave partial protection against dengue 1; unfortunately, the reaction of this serum against Ilheus could not be tested.

In summary, then, of the 11 acute sera used in

TABLE 4

Results of neutralization tests with acute and convalescent sera from cases of secondary infection with yellow fever

Case No.	Days after onset sera obtained		Virus strains used in tests											
			Dengue type 1				Dengue Tr 1751				Ilheus			
			Acute		Conval.		Acute		Conval.		Acute		Conval.	
	Acute	Conval.	AST	PR	AST	PR	AST	PR	AST	PR	AST	PR	AST	PR
Tr 4140	0	109	14.2	0/6	20.3	4/6								
Tr 4317	0	22	19.2	4/6	*21.0*	6/6							*13.7*	5/6
Tr 5689	12	36	18.3	3/6	*20.5*	5/6					*14.0*	6/6	*14.0*	6/6
Tr 5900	5	35	*21.0*	6/6	*21.0*	6/6					*14.0*	6/6	*14.0*	6/6
UR 11	−25*	28	14.3	1/6	19.5	4/6	9.5	0/6	14.0	1/6	6.5	0/6	10.5	3/6
UR 19	−16*	25	15.8	1/6	*20.7*	5/6	13.7	0/6	*17.0*	6/6			*14.0*	6/6
UR 48	3	47			*21.0*	6/6	10.6	0/6	15.5	3/6	6.7	0/6	7.7	0/6
UR 51	2	35	14.8	1/6	16.7	1/6	8.3	0/6	14.8	3/6	*14.0*	6/6	*14.0*	6/6
UR 79	1	31	16.5	0/6	*21.0*	6/6	9.5	0/6	15.5	2/6	*14.0*	6/6	*14.0*	6/6
AP 24	1	10			*20.3*	5/6	9.5	0/6	15.3	4/6			*14.0*	6/6
AP 96	3	26	13.5	1/6	17.5	2/6					7.0	0/6	7.5	0/6
AP 66	3	25											*14.0*	6/6

* Sera obtained for routine survey purposes before the onset of the yellow fever epidemic.

the protection tests, four give clear, and a fifth partial, evidence of a previous infection with a Group B virus. In evaluating the six negative cases, it should be borne in mind that only a limited number of viruses were used in the protection tests. The amount of serum available made it necessary to restrict its use to testing for viruses known to be active in the area. It is now known that at least one other Group B virus (St. Louis) may be found in Trinidad. In one of the negative cases (Tr 4140) and one of the doubtful cases (Tr 4317), it was not possible to test for antibodies against more than one virus.

We must, therefore, turn to the HI tests on acute sera for further evidence. Of the six sera that failed to provide protection against the Group B antigens used (Tr 4140, UR 11, UR 19, UR 48, AP 24 and AP 96), four (Tr 4140, UR 11, UR 19 and AP 24) inhibited agglutination with two or more of the Group B antigens. In one of them, the reaction occurred in only the first tube and may be nonspecific. If this test is accepted at face value, however, we are left with only two acute sera in group II (UR 48 and AP 96) that gave no evidence in either the protection or HI tests of previous Group B infection; acute serum from a third, AP 66, was simply not available for these tests. These three cases have been placed in group II largely because their convalescent sera fixed complement with Group B antigens according to the pattern typical of patients with a demonstrable previous infection with either Ilheus or dengue. It should be noted here that convalescent serum UR 48 protected against dengue, and convalescent serum AP 66 against Ilheus, as well as against yellow fever. As will be shown later, this early development of heterologous antibody is more characteristic of group II than of group I or primary infections.

The fact that no Group B antibodies of any kind could be demonstrated in the acute sera of the two out of these three cases tested may be interpreted in various ways. In the first place, it could be concluded that the individuals in question had never suffered from a Group B infection before succumbing to yellow fever. Such an assumption would, of course, cast doubt on the generality of the hypothesis that the broad pattern of heterologous immune response described as characteristic of our second group of yellow fever patients is due to sensitization by a previous attack of Group B infection. Somewhat more probable is the supposition that these patients had suffered a Group B infection by a virus (e.g., St. Louis) which was not used in our battery of tests, or that infection with Ilheus or dengue had occurred so long ago that demonstrable antibodies had disappeared.

Since either of these latter assumptions seems reasonable, it is proposed, for the time being at

least, to refer to the group II infections as secondary or sensitized infections and to regard the immunological patterns exhibited by group I as characteristic of infection in individuals who have not suffered a previous attack with a Group B virus, i.e., as primary infections.

We may now turn to a presentation of various other characteristics that differentiate the immune responses developed in the two types of infection.

TIME COURSE OF ANTIBODY PRODUCTION AND DISAPPEARANCE

Primary Infection

Table 1 summarizes the results obtained by HI, CF and yellow fever N tests in the 17 cases we consider to be primary infections of yellow fever.

The convalescent sera from all 17 cases inhibited agglutination with a yellow fever antigen and, in most instances, to a greater or lesser extent with other Group B antigens. These results indicate that, in primary yellow fever infections, homologous HI antibodies are produced first. Heterologous antibodies, however, appear very shortly afterwards. This order of development is illustrated particularly clearly by the findings with two convalescent sera from AP 55, obtained 11 days and 24 days after onset. In the HI tests, the first specimen gave a completely specific reaction, whereas the second inhibited agglutination with all four Group B antigens used in the test. Most of the late convalescent sera showed a very broad overlap with other Group B antigens. However, the titers obtained with a yellow fever antigen were invariably as high as, or higher than, those obtained with other antigens. Titers in general were moderate and ranged from 1:10 to 1:640. There is a marked tendency for titers to decline with time, as is clearly shown by the results obtained with the three convalescent sera from Tr 2942, secured 36, 103 and 441 days after onset. The HI titers with a yellow fever antigen were 1:640, 1:160 and less than 1:10, respectively. A similar decline was observed in most cases where two convalescent sera were available. That HI antibodies may develop very rapidly is shown by the studies of case Tr 4754; the acute serum obtained on the fifth day of disease inhibited agglutination with all four Group B antigens.

In primary infections, the CF test was remarkably specific. As a rule, cross-reactions with other Group B antigens occurred only when the homologous titer was high. Reactions with heterologous antigens were low. An exception to this pattern is case AP 53, in which the convalescent serum obtained 24 days after onset fixed complement in a 1:8 dilution with yellow fever and Ilheus antigens; a slight anticomplementary action of this serum could not be entirely ruled out.

There is some evidence that CF antibodies develop later than HI antibodies. In several instances, sera obtained soon after infection (11 days) were negative by the CF test even though positive by the HI (cases AP 22 and AP 55).

In the seven cases for which more than one convalescent serum was available, the HI test had reached maximum titers in the first specimen, taken between the 36th and 50th days (at 11 days for AP 55), and gave lower titers in the second specimen taken between the 59th and 293rd days. The CF titer, on the contrary was, with one exception (UR 57), greater in the second specimen than in the first.

There is great variation in the time after onset at which CF antibodies become demonstrable. The earliest bleeding giving a positive result was obtained on the fifth day after onset (case Tr 4754). Two sera (AP 55 and AP 22) obtained 11 days after onset were negative. A second convalescent serum from AP 55, obtained 24 days after onset, fixed complement in the presence of a yellow fever antigen in high titer. The 11th-day bleeding from AP 22 was the only convalescent serum available and consequently it is not known if or when the patient developed CF antibodies. In two instances (Tr 2942 and UR 29), sera obtained as late as 36 and 29 days after onset showed only a trace of CF antibodies. Bleedings from these patients obtained 103 and 59 days after onset, however, fixed complement in dilutions of 1:16 and 1:64, respectively. These results indicate that CF antibodies may on occasion be slow in developing, but are produced over a long period of time. In fact, CF antibodies were usually found to be increasing at a time when HI antibodies were decreasing.

As will be shown later, the pattern of CF antibodies in primary cases of yellow fever differs markedly from that seen in secondary infections. Our studies indicate that the CF test with a battery of Group B antigens is the method of

choice for distinguishing primary from secondary infection.

As determined by the intracerebral test in adult mice, the time needed for development of specific N antibodies following a primary infection, varies considerably. Antibodies may be present as early as the fifth day (Tr 4754). On the other hand, their development may be very slow. The results given by the two convalescent sera from case Tr 2942 are a good illustration of delayed appearance of antibodies. The first convalescent serum, obtained 36 days after onset, gave a negative result, whereas the second, obtained after 103 days, gave clear-cut protection. Several of the convalescent sera obtained up to 50 days after onset gave inconclusive or partial protection in the standard intracerebral yellow fever N tests.

Secondary Infections

The pattern of HI antibodies in what we interpret as secondary infections differs radically, as a rule, from that seen in primary infections. Table 2 shows that there was usually no evidence of relative specificity. The titers obtained with all antigens were almost always high, ranging from 1:640 to more than 1:20,480. The CF results were equally nonspecific. In most instances, the titers obtained with heterologous antigens were higher than those obtained with a yellow fever antigen. The available evidence indicates that, in all probability, the majority of the cases classified as secondary infections were instances of yellow fever in Ilheus-immune individuals. In the analysis of the CF patterns obtained with the convalescent sera the fact is brought out that the titers obtained with an Ilheus antigen were invariably as high as, or higher than, the titers observed with the other Group B antigens included in the test. Almost equally high titers were obtained with a St. Louis antigen; results no doubt due to the marked immunological overlap between this virus and Ilheus.

There was some indication that in secondary infections both the HI and CF antibodies appeared earlier than in primary infections. This pattern is clearly illustrated by the results obtained with sera from cases AP 22, a primary infection, and AP 24, a secondary infection. With the convalescent serum from AP 22, taken 11 days after onset, the only reaction observed in the HI test was a 1:20 inhibition with a yellow fever antigen; the CF test was completely negative. The 10th-day serum from AP 24 reacted in the HI tests in titers of 1:2,560 to 1:20,480 with six different Group B antigens and in the CF test with titers of more than 1:128 with the four antigens used. The acute specimen from AP 22 was completely negative by all tests, while the acute specimen from AP 24 gave a 1:20 inhibition with an Ilheus antigen. Furthermore, the convalescent specimen obtained from AP 24 showed protective antibodies in an Ilheus N test. The indications are that AP 24 is an example of yellow fever infection in an Ilheus-immune individual. The fact that in the standard yellow fever protection test the convalescent serum from AP 22 gave an inconclusive result whereas that from AP 24 was fully protective, may also be significant as indicating more rapid production of yellow fever N antibodies in the latter than in the former.

Hemagglutination-inhibiting antibodies were present in six of nine acute sera and in two preinfection sera from cases of secondary infection. Since four of the positive acute sera were obtained during the first few days after onset of illness, the HI antibodies can be taken as good evidence of previous Group B virus infections. The HI antibodies in the other two positive acute sera (Tr 5900 and Tr 5689), obtained on the fifth and twelfth days, could be the result of the yellow fever infection. No HI studies were made on one of the acute specimens (AP 66).

Not enough observations are available to determine the duration of the pattern of HI and CF antibodies following a secondary infection.

In general, the development of yellow fever neutralizing antibodies was more marked in secondary infections than in primary infections. All but two of the convalescent sera gave clear-cut protective results. The two exceptions were a 36th-day specimen from Tr 5689 and a 25th-day specimen from AP 66, both of which gave only partial protection. Tr 5689 is of unusual interest. The acute serum, which was obtained on the twelfth day of disease (Downs *et al.*, 1955), not only had high titers of HI antibodies to all Group B agents, but also gave clear-cut protection results in Ilheus and partial protection in dengue type 1 N tests. This case is manifestly an example of secondary infection. In the yellow fever N test, however, both the acute and convalescent sera from Tr 5689 were only slightly protective. The acute specimen gave a 2/6 protective ratio with

an AST of 7.5 days, and the convalescent serum gave a similar protective ratio but an AST of 8.7 days.

THE DEVELOPMENT OF HETEROLOGOUS NEUTRALIZING ANTIBODIES

The results of the in vitro tests indicated clearly that, following a yellow fever infection, antibodies are developed that react not only with an homologous antigen but also with related Group B antigens. These cross-reactions were much more marked in the cases we have classified as secondary infections. It was of interest to determine if N antibodies also gave patterns characteristic for the two types of infection. The viruses used in the tests were dengue type 1, Tr 1751 strain of dengue type 2 and Ilheus. Results for primary infections are summarized in Table 3, and those for secondary infections in Table 4.

The results show that in cases of primary infection N antibodies to the two types of dengue were entirely absent in all acute sera. They also suggest strongly that, following a primary yellow fever infection, there is a development of minimal amounts of N antibodies to the two types of dengue. The AST's obtained with the convalescent sera were in general longer than the AST's for the acute sera. Only two convalescent sera gave partial protection results, in both instances against dengue type 1 virus: a 50th-day specimen from case Tr 4403 and a 40th-day specimen from UR 220. The acute serum from case Tr 4403 was negative in the test; the acute serum from case UR 220 was not tested. All of 11 acute or preinfection sera and 23 convalescent sera from primary infections gave completely negative results in the Ilheus N test.

The pattern of N antibodies in secondary infections was quite different from that in primary infections (Table 4). In the dengue type 1 test, one acute serum (Tr 5900) gave a positive result and two (Tr 4317 and Tr 5689) gave partial protection. These three cases may have had a previous dengue infection. The remaining six preinfection and acute sera were negative. Of eleven convalescent sera tested, seven were protective, two inconclusive and two negative to dengue type 1. It will be observed that whenever paired sera from the same individual were tested, in all cases but one (UR 51) the AST's of the convalescent sera were significantly longer than those of the acute-phase sera. These results indicate clearly that a yellow fever infection in the majority of these cases led to the development of significant amounts of antibodies capable of neutralizing dengue type 1 virus.

Although the number of tests made with the TR 1751 strain of dengue type 2 is limited, it is clear that all of six convalescent sera showed some ability to neutralize this virus, in one instance (UR 19) sufficient to protect all the mice. In addition, three of these sera gave partial protective results.

In the Ilheus N tests, four of seven acute sera and eight of eleven convalescent sera were protective. One convalescent serum was partially protective and two were negative. Paired sera from seven patients were available. In four instances, both members of a pair protected the mice; an indication that the donors of these sera were Ilheus-immune at the time they acquired their yellow fever infection. Of the remaining three pairs, all three acute and two convalescent sera were negative in Ilheus protection tests; the third convalescent serum gave partial protection. The positive Ilheus convalescent sera can be taken as good evidence of a secondary infection. It should be recalled here that all the convalescent sera from primary infections were negative in the Ilheus N tests. We are probably correct in concluding that the majority of the cases which we have classified as secondary infections were in fact cases of yellow fever infection in Ilheus-immune individuals.

DISCUSSION

The clear distinction in antibody patterns between primary and secondary infections with yellow fever may be considered typical of infections with Group B agents. Evidence for this statement has been derived in these laboratories from the results of experiments in animals with a variety of Group B viruses as well as from the study of St. Louis and Japanese B infections in man. The marked serological overlaps shown by convalescent sera are obviously very complicating factors in the specific diagnosis of Group B infections. Our results indicate clearly that before the serological reactions can be interpreted, it is essential to determine whether the infection is primary or whether it is a superinfection in an individual who has previously had a Group B infection. In primary cases of yellow fever the development of N antibodies, as determined by

the standard intracerebral test, is quite specific. However, with other Group B agents such as West Nile, for example, heterologous N antibodies may develop.

The fact that an infection is indeed a primary one can be determined only by CF and HI tests with a battery of antigens prepared from a variety of Group B agents. In CF tests with sera from primary infections, the highest titers are invariably given with homologous antigens; in fact, in yellow fever infections the CF test may at times be completely specific. Heterologous CF antibodies, if they develop, do so always to a much lower titer than the homologous. In secondary infections, there is a very rapid and massive development of HI and CF antibodies to a great variety of related agents. Our limited experience indicates that a stimulation of N antibodies likewise may occur. When the serological response of a secondary infection is observed, the only legitimate conclusion is that the infection is due to a Group B virus; no specific diagnosis is possible. In such a case, only the isolation and identification of the virus will permit a specific diagnosis to be made.

Our studies indicate that in primary infections HI antibodies develop before CF or N antibodies. The first HI antibodies to appear in convalescence are specific, followed very rapidly by heterologous antibodies. It seems probable that in primary infections HI tests may give a truly specific diagnosis if applied to early bleedings taken before CF or N antibodies have developed. HI antibody studies are also of value in distinguishing between primary and secondary infections; the patterns developed are not as distinctive, however, as those observed in CF tests.

Obviously, the demonstration of a rise of antibodies by any test in which only one virus (or antigen) is used, is not conclusive evidence that the infection was due to that particular virus. The test must include all Group B viruses (or their corresponding antigens) known to be present in the region. In regions such as California or Egypt, where only one Group B virus is known to infect man, tests with only one agent would appear to be adequate; with St. Louis virus in California and West Nile in Egypt. Even here, however, the possibility should not be ignored that other, at present unknown, Group B viruses may be present.

SUMMARY AND CONCLUSIONS

1) The serological responses of 29 cases of human yellow fever infection were studied by hemagglutination-inhibition (HI), complement-fixation (CF) and neutralization (N) tests.

2) The cases were divided into two groups, chiefly according to the pattern of CF antibodies to a variety of Group B agents. The 17 cases in the first group are considered to be primary infections of yellow fever; the 12 cases in the second are considered to be infections of yellow fever in individuals who had previously been infected with another Group B virus.

3) In primary infections of yellow fever specific HI antibodies appear first, followed rapidly by antibodies to other Group B viruses. The titers of homologous HI antibodies are always as high as, or higher than, the titers of heterologous antibodies.

4) In primary infections there is great variation in the time after onset at which CF antibodies become demonstrable. Complement-fixing antibodies are produced over a long period and may still be increasing when HI antibodies are diminishing. The pattern of CF antibodies is remarkably specific. Overlaps with other antigens occur only when the homologous CF antibodies are high.

5) In primary infections the development of N antibodies as determined by the standard intracerebral test is quite specific. Heterologous N antibodies are developed to a limited extent.

6) In cases of secondary infection, the production of HI and CF antibodies is both massive and very rapid. There is no suggestion of specificity in the patterns developed and, in fact, as a rule the highest HI and CF antibodies were heterologous. Furthermore, in secondary infections the production of heterologous N antibodies is stimulated to a greater extent than in primary infections. In the present 12 cases, the heterologous response was so great that in all but one of the cases accurate diagnosis of the current illness by N test was impossible.

REFERENCES

CASALS, J., 1957. The arthropod-borne group of animal viruses. *Trans. N. Y. Acad. Sci.*, Ser. II, **19**: 219–235.

CASALS, J., AND BROWN, L. V., 1954. Hemagglutination with arthropod-borne viruses. *J. Exp. Med.*, **99**: 429–449.

CASALS, J., AND PALACIOS, R., 1941. The complement-fixation test in the diagnosis of virus

infections of the central nervous system. *J. Exp. Med.*, **74:** 409–426.

CLARKE, D. H., AND CASALS, J., 1955. Improved methods for hemagglutination studies with arthropod-borne viruses. *Proc. Soc. Exp. Biol. and Med.*, **88:** 96–99.

CLARKE, D. H., AND THEILER, M., 1955. The hemagglutinins of Semliki Forest and Bunyamwera viruses, their demonstration and use. *J. Immunol.*, **75:** 470–474.

DOWNS, W. G., 1955. Epidemiological notes in connection with the 1954 epidemic of yellow fever in Trinidad, B.W.I. *Yellow Fever. A Symposium in Commemoration of Carlos Juan Finlay.* The Jefferson Medical College of Philadelphia. Pp. 71–75.

DOWNS, W. G., ANDERSON, C. R., AND SPENCE, L., 1955. The isolation of yellow fever virus from a human patient on the twelfth day of illness. *Trans. Roy. Soc. Trop. Med. and Hyg.*, **49:** 577–579.

SABIN, A. B., 1951. Hemagglutination by viruses affecting the human nervous system. *Fed. Proc.*, **10:** 573–578.

SABIN, A. B., AND BUESCHER, E. L., 1950. Unique physico-chemical properties of Japanese B encephalitis virus hemagglutinin. *Proc. Soc. Exp. Biol. and Med.*, **74:** 222–230.

SWEET, B. H., CHANOCK, R. M., AND SABIN, A. B., 1953. Recovery and characterization of hemagglutinins from two immunologically distinct types of dengue virus. *Fed. Proc.*, **12:** 462.

A NEW MONOPHASIC MEDIUM FOR THE CULTIVATION OF *ENTAMOEBA HISTOLYTICA*

RALPH E. DOLKART[1] AND BERNARD HALPERN[2]

Culture media capable of supporting the growth of *Entamoeba histolytica* have been adequately reviewed by Anderson *et al.*, (1953), Rees (1956), and Balamuth and Thompson (1955). Anderson *et al.*, (1953) tabulated some of the more recent innovations concerning the growth requirements of *E. histolytica* in media. The majority of these media, as well as many described by earlier investigators, contain a complexity of ingredients which are difficult to prepare and in some instances unstable upon storage.

During the course of experimental investigations designed to cultivate *E. histolytica* without concomitant growth of a living associate, numerous media have been employed by ourselves as well as others without success. On the basis that the primary site of invasion for *E. histolytica* in man is the intestinal tract, that the major involvement occurs in the mucous membrane and that the characteristic substance thereon is mucus, we investigated the possibility that mucus might contain some elements which, in whole or in part, would support the growth of *E. histolytica*.

Morita (1938), interested in demonstrating cytolytic effects of living *E. histolytica*, suspended uniform cell suspensions of mucous membranes of different animals in an ameba culture. He reported that cell suspensions containing amebas had fewer cells than those without amebas and concluded that *E. histolytica* has a definite cytolytic effect.

Powdered gastric mucin has been used in the past as a selective agent for enhancing the invasive activity of bacteria in vivo; (McLeod, 1944; Nungester *et al.*, 1932; Olitski, 1948) and has been incorporated into media on the supposition that it is a stabilizer of bacterial growth in vitro.

[1] Parmly Research Laboratory of The Department of Medicine, Northwestern University Medical School.

[2] Department of Microbiology, Northwestern University Medical School.

MATERIALS AND METHODS

A. Culture medium.

gastric mucin powder[3]	5 gm
distilled water	100 ml

This mixture was autoclaved for 15 minutes at 15 pounds pressure, cooled and centrifuged for 15 minutes at 2,000 rpm. The supernatant was then removed and saved as the stock gastric-mucin medium.

The stock medium was further processed in either of two ways; 1) the stock gastric-mucin medium was diluted to 1% with 0.8% NaCl solution, and 0.5 ml of Balamuth's medium (1946) was then added for each 2.5 ml of the 1% gastric-mucin medium; 2) the stock gastric-mucin medium was diluted to a 1% concentration with sterile M/15 or M/30 K_2HPO_4-KH_2PO_4 containing 0.8% NaCl.

These preparations were placed in tubes in 3.0-ml amounts and a small amount of rice starch (Stein Hall) was added prior to inoculation with amebas and bacteria.

B. Ameba strains. A U.C. strain[4] of *E. histolytica* which had been growing with a multibacterial flora in Balamuth's medium in our laboratory for 7 years, and a more recently acquired strain of *E. histolytica* (200[5]), also growing with a mixed bacterial flora, were employed for testing the growth-promoting activity of the gastric-mucin media. The 200 strain had been carried on Locke egg-serum medium.

Transfers of these ameba strains were made from their prior accustomed medium into gastric-mucin medium and incubated at 37°C for 48 or 72 hours. A schedule fitted into the normal working week (Mon., Wed., Fri.) was used for transfer of amebae to fresh medium.

[3] Wilson #440.

[4] U.C. strain refers to a strain of *E. histolytica* isolated at the University of Chicago a number of years ago.

[5] The 200 strain of *E. histolytica* was received from the National Institutes of Health at Bethesda, Md.

RESULTS

For a period of 1 year, *E. histolytica* strains U.C. and 200, with multibacterial flora grown in gastric-mucin medium with Balamuth medium additive and transferred according to the schedule previously given, consistently yielded cultures containing numerous viable amebas as evidenced by microscopic examination. Homogenizing the fluid culture medium of random samples containing independently-growing cultures of *E. histolytica*, strains U.C. and 200 with mixed bacterial flora, resulted in counts of 20,000 to 30,000 amebas per ml, on the average, for both strains. Balamuth medium in saline did not support the growth of amebas when 0.5 ml of this medium was added to 2.5 ml saline.

One per cent gastric mucin in M/15 or M/30 phosphate buffer, has supported the growth of the U.C. strain of amebas for 4 months, whereas the 200 strain during this period grew in the expected range (20,000 to 30,000 per ml) in the M/30 preparation but did not grow in the M/15. Nonbuffered gastric-mucin medium without basal medium additive supported growth of the amebas for 3 to 4 transfers only.

SUMMARY

A simple medium prepared from powdered gastric mucin is described for the cultivation of *Entamoeba histolytica*.

Excellent growth of two strains of *E. histolytica* grown with bacterial associates was maintained in this medium.

REFERENCES

ANDERSON, H. H., BOSTICK, W. L. AND JOHNSTONE, H. G., 1953. *Amebiasis-Pathology, Diagnosis and Chemotherapy*. Springfield, Ill., Charles C Thomas.

BALAMUTH, W., 1946. Improved egg yolk infusion for cultivation of Entamoeba histolytica and other intestinal protozoa. *Am. J. of Clin. Path.*, **16:** 380.

BALAMUTH, W., AND THOMPSON, P. E., 1955. In *Biochemistry and Physiology of Protozoa*. Vol. 2, by S. H. Hutner and André Lwoff. New York, Academic Press, Inc.

MCLEOD, C., 1941. The mode of action of mucin in experimental meningococcus infection; II. The effect of mucin upon the defense mechanism of the mouse. *Am. J. Hyg.*, **34** (Sect. B.): 51.

MORITA, Y. J., 1938. Investigation on amoebic dysentery: V. On cytolytic activity of dysenteric amoebae and on test against tissular invasion in vitro. *Orient. Med.*, (Abstr. sect.) **28:** 40.

NUNGESTER, W. J., WOLF, A. A., AND JOURDONAIS, L. F., 1932. Effect of gastric mucin on virulence of bacteria in intraperitoneal injections in the mouse. *Proc. Soc. Exp. Biol. and Med.*, **30:** 120.

OLITSKI, L., 1948. Mucin as a resistance lowering substance. *Bact. Rev.*, **12:** 149.

REES, C. W., 1956. *Problems in Amoebiasis*. Springfield, Ill., Charles C Thomas.

THE FIRST CASE OF SPARGANOSIS IN COLOMBIA

DAVID BOTERO AND JOSE J. GOMEZ

School of Medicine, University of Antioquia and San Vicente Hospital; Medellín, Colombia

Human sparganosis, infection with plerocercoid larvae of *Diphyllobothrium* species, subgenus *Spirometra*, was first reported by Sir Patrick Manson in China in 1882. Since then many cases have been found in several countries, especially in the Far East (Weinstein *et al.*, 1954; Yogore and Tangco, 1953; Sandars, 1954). The first case in North America, according to Mueller and Coulston (1941) was reported by Stiles in 1908. The second was reported by Moore (1915). About ten more cases have been described from the United States since that time. In South America the first case was found by Daniels in British Guiana in 1910 and the second in Uruguay by Osimani and Peyrallo (1954). The present case is the third in South America and the first in Colombia (Neghme, 1958, personal communication; Basnuevo, 1958, personal communication; León, 1958, personal communication; Maldonado, 1958, personal communication; Osimani, 1958, personal communication).

CLINICAL RECORD

On September 6, 1957 there was admitted to the Eye Service of San Vincente Hospital in Medellín, a white girl, age 18, coming from a temperate rural area, where she lived under very poor hygienic conditions and in close contact with domestic animals: dog, cat, pigs, chickens, etc. The diet was protein-deficient. The raw water she drank came from an open spring. She complained of protrusion of the left eye, palpebral edema, burning sensation and feeling of a foreign body. The evolution of the disease was 1.5 years, during which time the symptoms appeared intermittently. There was no impairment of vision. Physical examination revealed only the lesion of the left eye and chronic tonsilitis. The exophthalmus was remarkable, with congestion of the conjunctiva and edema of the upper eyelid. In the upper nasal angle a lump, creamy in color, about 3 cm in diameter, was visualized, surrounded by a hyperemic area. The visual acuity was 20/20. Tonometry was 18 mm (Schiotz). The fundi were normal. X-rays of the skull, optical cavities and paranasal sinuses were normal. Laboratory examinations showed normal blood chemistry, negative Wassermann, and *Ascaris lumbricoides* and *Trichuris trichiura* in the stools. Hematological findings were: red blood cells, 4,800,000; hemoglobin, 13 gms%; white blood cells, 12,500, with 70 per cent neutrophils, 6 per cent eosinophils and 24 per cent of lymphocytes.

The eye nodule was punctured, and a flat, whitish, nonmotile worm, with the appearance of a tapeworm, was removed by traction. Next day the lesion was opened surgically and only the capsule surrounding the cavity left by the parasite was found. Histological sections showed connective tissue with inflamatory exudate and eosinophils. The patient recovered almost completely from her ocular difficulty. The blood count showed a diminution of eosinophils from 6 per cent to 1 per cent.

DESCRIPTION OF THE PARASITE

The worm measured 9 cm long by 3 mm wide; it was wrinkled more or less transversely, flat, whitish, and without any differentiated scolex (Fig. 1). After fixation in 10 per cent formalin it was studied microscopically. A very thin cuticular membrane could be recognized. There were no internal organs or head but a large number of round and polyhedral bodies about 10μ in diameter, filling the whole body matrix (Fig. 2). These objects were interpreted by Dr. Ernest C. Faust (1958, personal communication), who identified the sparganum, as calcareous corpuscles of calcium carbonate that dissolve easily in acids and probably serve as buffer when the medium is acid.

COMMENTS

The adult stage of *Diphyllobothrium* species producing sparganosis in man lives in the intestinal tract of certain mammals. One of the commonest is *D. mansoni*, which utilizes dogs and cats as definitive hosts. The first intermediate host is a *Cyclops* and the second hosts are such vertebrates as frogs, snakes, birds and mammals (Faust, 1955). The life cycle has been studied by several authors (Mueller, 1938 a, b; Mueller and Coulston, 1941; Bonne, 1942), and a great variety

of animals have been infected with the sparganum stage. These experiments include man as intermediate host.

Yokogawa and Kobayashi (1930) point out that human infections result from a) ingestion of the infected *Cyclops* in raw water, b) eating raw flesh of the second intermediate host (frogs, snakes, etc.), which is a common practice in Korea, according to Weinstein *et al.*, (1954), and c) applying infected meat from the intermediate host as poultices on inflamed skin or mucous membranes. This is not rare in Colombia, where there is the popular custom of rubbing frogs for the treatment of erysipelas.

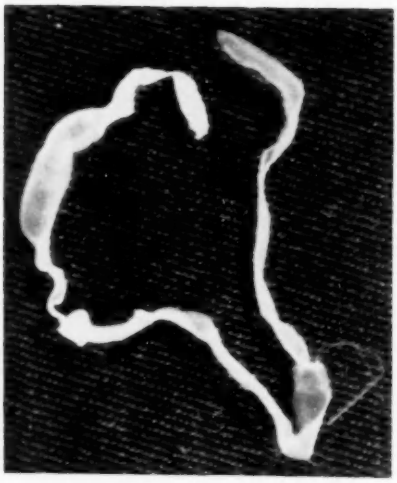


FIG. 1. Sparganum obtained from the eye of the patient after fixation in 10% formalin.

The patient harboring the sparganum described had never been out of Colombia. She denied having used frogs, reptiles or other animals as poultices on any part of her body. She usually ate well-cooked meat. The remaining possibility of acquiring the infection is the ingestion of infected *Cyclops* in raw water.

We made a field study directed toward finding the adult parasite in the vertebrates present in the patient's home. For this purpose we examined the stools of a cat, dog, cow, horse and pig. All were negative for *Diphyllobothrium* eggs. We also searched for *Cyclops* in the water the patient used for drinking, and looked for plerocercoid larvae in several frogs. These attempts were all negative. For this reason we were not able to identify the species to which the sparganum belongs.

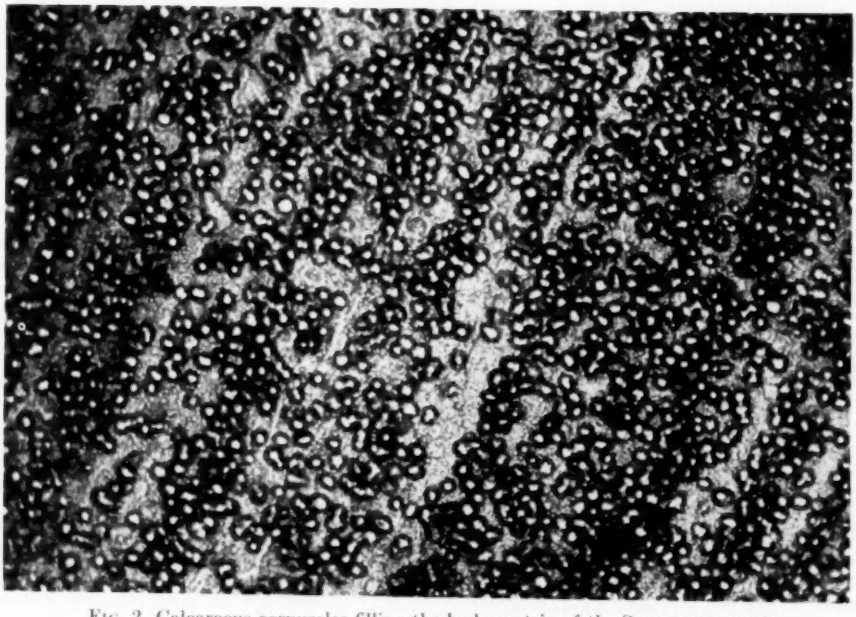


FIG. 2. Calcareous corpuscles filling the body matrix of the Sparganum, ×10

The ocular localization of human sparganosis appears to be very rare in the Western Hemisphere. We could find no reported case in the Americas. Houdemer (1934), described several cases in Indochina, where it is relatively common because of the custom of applying frogs as poultices to inflamed eyes. Joyeux *et al.*, (1939) reported 14 cases from Tonkin and described the most common localization of the spargana as the eyelids, under the conjunctiva, retrobulbar and periocular. Penetration of the sparganum into the eye ball has not been reported.

SUMMARY

The first case of sparganosis in Colombia has been found, being the third in South America. This seems to be the first of ocular localization to be reported in the Western Hemisphere. Investigations directed toward finding the adult parasite and larvae of *Diphyllobothrium* species in several animal hosts were negative.

ACKNOWLEDGMENT

We are deeply indebted to Dr. Ernest Carroll Faust who helped us in the identification of the sparganum and in the correction of the manuscript.

REFERENCES

Bonne, C., 1942. Researches on sparganosis in the Netherlands East Indies. *Am. J. Trop. Med. & Hyg.*, **22:** 643–645.

Faust, E. C., 1955. *Animal Agents and Vectors of Human Disease*. Lea & Febiger, Philadelphia.

Houdemer, Dodero, et Cornet, 1934. Les sparganoses animales oculaires en Indo-Chine et la sparganose. *Ann. d'Occul.* **171:** 311–338.

Joyeux, B., Cam-Cong, T., et Xuan-n, 1939. Nouvelles contributions a l'étude de la sparganose oculaire au Tonkin. *Rev. Med. Franc. Extreme Orient*, **17:** 27–46.

Manson-Bahr, F. H., 1948. *Enfermedades Tropicales*, 12a. Ed., Salvat Editores, Barcelona, Buenos Aires.

Moore, J. T., 1915. *Sparganum mansoni*. First reported American case. *J. Trop. Dis. & Prev. Med.*, **2:** 518–529.

Mueller, J. F., 1938a. The history of *D. mansonoides* Mueller, 1935, and some considerations with regard to sparganosis in the United States. *Am. J. Trop. Med.*, **18:** 41–66.

Mueller, J. F., 1938b. Studies on *Sparganum mansonoides* and *Sparganum proliferum*. *Am. J. Trop. Med.*, **18:** 303–328.

Mueller, J. F. and Coulston, F., 1941. Experimental human infection with the sparganum larva of *Spirometra mansonoides* (Mueller, 1935). *Am. J. Trop. Med.*, **21:** 399–425.

Osimani, J. J., y Peirallo, R., 1954. Segundo caso de sparganosis encontrado en America del Sur. Primer caso descrito en el Uruguay. *Arch. Urug. Med. Cir. y. Espec.*, **44:** 10.

Sandars, D. F., 1954. A sparganum from a Queensland woman. *Med. J. Australia*, **2:** 817–818.

Weinstein, P. P., Krawczyk, H. J., and Peers, J. H., 1954. Sparganosis in Korea. *Am. J. Trop. Med. & Hyg.*, **3:** 112–129.

Yogore, M. G., and Tangco, A. F., 1953. Notes on sparganosis on a Filipino. *Acta Med. Philippina*, **9:** 301–303.

Yokogawa, S., and Kobayashi, H., 1930. On the species of *D. mansoni* sensu lato, and on the infectious mode of human sparganosis. *Trans. 8th. Congres Far East Ass. Trop. Med.*, **2:** 215–226.

THE USE OF DITHIAZANINE IN THE TREATMENT OF HELMINTHIASIS IN MEXICAN FARM LABORERS[1]

E. D. WAGNER, F. R. LEMON, AND H. S. BURNETT

Department of Microbiology and Department of Preventive Medicine and Public Health, College of Medical Evangelists, Loma Linda, California

Recent reports (Bueding and Swartzwelder, 1957; Frye *et al.*, 1957; Swartzwelder *et al.*, 1957) indicate that a new experimental drug, dithiazanine iodide (Eli Lilly), is highly effective as a therapeutic agent against a number of helminth infections in man. Of particular interest is its apparent effectiveness in the treatment of trichuriasis (whipworm infection) and strongyloidiasis (*Strongyloides stercoralis* infection), two worms for which no satisfactory treatment had previously been available.

Frye *et al.*, (1957) report a cure rate of 87.5 per cent of the 16 patients treated for *Trichuris trichiura*, on a dosage schedule of 200 mg of dithiazanine three times a day (t.i.d.) for 5 days, and a 71 per cent cure rate of 17 patients treated on a dosage schedule of 200 mg twice daily (b.i.d.) for 5 days. The drug was administered orally. No significant signs of intolerance to the drug were reported. Activity against *Ascaris lumbricoides* was noted in the study. Bueding and Swartzwelder (1957) report a cure rate of 89 per cent of 18 cases of strongyloidiasis, based on negative findings in stool and duodenal drainages after treatment, on a dosage schedule of 200 mg of the compound t.i.d. for from 5 to 21 days.

Swartzwelder *et al.*, (1957) report 97 per cent of 164 patients cured of trichuriasis when treated with doses of 200 mg t.i.d. for 5 days. Marked egg-count reduction in ascariasis was observed in 97 per cent of 42 persons treated with up to 600 mg daily in divided doses for 5 days. Twenty-eight of these persons showed complete elimination of the infection. Sixteen of 21 patients (also reported by Swartzwelder, *et al.*, 1958) were cured of strongyloides when treated for 5 to 21 days. A dosage schedule of 100 mg of dithiazanine b.i.d. reportedly cured all of 35 cases of enterobiasis (also reported by Miller, *et al.*, 1958). Some egg reduction was effected in hookworm infections by the use of the drug but better results were obtained when subcurative doses of tetrachlorethylene were given with the drug. All of the above studies were made in the United States, although one report includes results obtained on patients in Costa Rica.

The present paper gives data obtained on the use of dithiazanine given orally for the treatment of helminthic infections in Mexican laborers employed by the Imperial Valley Farmers' Association in California. The arrangement provided the advantages of a reasonably high infection rate among persons to be studied within the continental United States, and a low probability of reinfection, since these individuals were removed from their previous endemic environments in Mexico. It also provided active out-patients rather than hospitalized individuals. A disadvantage, however, was the difficulty encountered in making follow-up studies due to the frequent moving of patients to different farms and camps. Of 1,006 individuals examined, 91 were found to be positive for one or more helminth infections, an infection rate of 9 per cent. Of these, 64 could be followed adequately after treatment for the purposes of this report. All pre- and post-therapy stool specimens were concentrated by the zinc sulfate flotation method.

Two dosage schedules were given about equally in the cases treated. Schedule No. 1 provided 200 mg t.i.d. for 4 days, or a total of 2,400 mg of dithiazanine. In Schedule No. 2 the patients received 300 mg t.i.d. for 3 days, or a total of 2,700 mg. Doses used by other investigators have averaged 600 mg per day for 5 days, or 3,000 mg total, or more, in some cases. The average weight of the individuals in this

[1] This investigation was supported by a grant from Eli Lilly and Company, who also provided the drug, Dithiazanine (Compound No. 01748).

Grateful acknowledgement is made to John Ariaudo, M.D. and the Imperial Valley Farmers' Association of El Centro, California for their cooperation and provision for laboratory facilities, and to Dora Rivera for assistance in the laboratory and as interpreter.

study was 137 pounds, ranging from 111 to 187 pounds. This is probably lower than for those of other adult study groups in the United States.

In order to observe for toxic effects, urine and blood studies were conducted on 13 cases before and after treatment. The urine was examined for the presence of albumen, hemoglobin, and organized sediment. Studies on the blood consisted of hemoglobin determination, white cell count, and differential count. No significant changes were observed in tests made up to 1 week after treatment. However, there were some reports of nausea by the patients. Of 73 persons medicated, 24 (32.8%) complained of nausea, while 18 (24.7%) complained of nausea and vomiting. This phase of the study was complicated by an outbreak of food poisoning in one of the main camps where these patients were housed. To what extent this distress was due to the medication and how much to the epidemic could not be determined.

One of the patients, convalescing from an attack of hepatitis, was unable to tolerate the drug. In another instance the drug was tolerated only in dosages of 100 mg t.i.d. In this case, the treatment was continued until 2,400 mg had been given. An apparent cure of hookworm infection resulted.

The environmental temperature and the type of work done by the men examined apparently had some influence on the side effects produced as, on the average, more frequent and more

TABLE 1

Final findings after treatment with dithiazanine

Infection	Cases treated and rechecked	Negative after treatment	
		No.	Percent
Ascaris	22	22	100.0
Trichuris	5	5	100.0
Hookworm	16	3	18.7
H. nana	23	10	43.5
Taenia sp.	6	6	100.0
E. vermicularis	3	3	100.0
Strongyloides	1	1	100.0

TABLE 2

Final status of each infection

	Ascaris	Trichuris	Hookworm	Strongyl	Pinworm	H. nana	Taenia	Total
Infections	27	7	23	1	3	36	7	104*
Infections not treated	2	0	4	0	0	5	0	11
Infections treated but lost before recheck†	3	2	3	0	0	8	1	17
Infections treated and rechecked	22	5	16	1	3	23	6	76
Schedule no. 1	11	2	9	1	2	11	3	39
Cases closed								
after 1 neg. recheck	4	0	0	0	1	2	1	8
after 2 neg. rechecks	6	2	1	0	0	2	1	12
after 3 neg. rechecks	1	0	0	1	1	0	1	4
Positive on first recheck	0	0	6	0	0	6	0	12
after 1 neg. recheck	0	0	2	0	0	1	0	3
after 2 neg. rechecks	0	0	0	0	0	0	0	0
Schedule No. 2	11	3	7	0	1	12	3	37
Cases closed								
after 1 neg. recheck	5	1	0	0	1	2	1	10
after 2 neg. rechecks	6	1	1	0	0	3	1	12
after 3 neg. rechecks	0	1	1	0	0	1	1	4
Positive on first recheck	0	0	4	0	0	4	0	8
after 1 neg. recheck	0	0	1	0	0	1	0	2
after 2 neg. rechecks	0	0	0	0	0	1	0	1

* 104 infections less 11 (double infections) and 2 (one triple infection) = 91 individuals represented.

† Cases were lost due to repatriation or inability to re-establish contact with patient.

severe complaints were received during hot days than cool days. It was further noted that practically no complaints came from men who did not work directly exposed to the hot sun.

The follow-up stool examinations were made at approximate intervals of 10 days, 30 days, and 90 days following therapy. In some cases it was possible to obtain only 1 or 2 post-treatment stools for examination. The results are given as positive or negative for ova, and are summarized in Table 1. All of the five cases of trichuriasis and the single case of strongyloidiasis were negative for check periods up to three months. All 22 cases of ascariasis remained negative for eggs after treatment as did the three cases of enterobiasis.

The results obtained in the treatment of 16 cases of hookworm infections are about as reported by other workers. In only three cases did the stools remain negative for the duration of the follow-up periods of 55 days, 69 days and 78 days.

Of additional interest is the activity of the drug observed in treating 23 cases of *Hymenolepis nana* and 6 cases of *Taenia* sp. infections. Ten (43.5%) of the 23 *H. nana* infections were, by the criteria given, successfully treated with dithiazanine and all of the six cases of taeniasis remained negative during the varying follow-up examinations made. The use of dithiazanine for treating tapeworm infections has not previously been reported.

For a detailed account of the dosage schedule used, the number of follow-up examinations made, cases which became positive after one or more negative findings, etc., Table 2 is given.

SUMMARY AND DISCUSSION

Two dosage schedules of dithiazanine, 200 mg t.i.d. for 4 days and 300 mg t.i.d. for 3 days were given Mexican farm laborers infected with certain helminths. Post-therapy stool studies revealed apparent cures in all patients infected with five species of helminths, as follows: Twenty-two cases were treated and found negative on subsequent stool examinations for *Ascaris lumbricoides*; similarly five for *Trichuris trichiura*; six for *Taenia* sp.; three for *Enterobius vermicularis*; and one for *Strongyloides stercoralis*. Results on other infections treated are: Ten of 23 cases with *Hymenolepis nana* and three of 16 hookworm infections remained negative for eggs.

The results of the study, based on active out-patients treated with dithiazanine, agree with those of others indicating quite effective therapeutic results obtained in treating trichuriasis and strongyloidiasis, as well as ascariasis and enterobiasis. The drug is less effective against hookworm infection. Of additional interest is the report of apparent drug activity against two tapeworms, *H. nana* and *Taenia* sp., findings not previously reported. In 10 of 23 (43.5%) of the *H. nana*-positive cases treated, the stools remained negative for eggs of this worm. All six of the cases of taeniasis remained negative for the duration of the test period.

Urine was examined before and after treatment in 13 cases and no significant changes were noted; likewise, blood hemoglobin and differential counts were made on the same cases, the findings indicating no significant change after use of the drug. Of 73 treated cases, 32.8 per cent reported some nausea and 24.7 per cent vomiting during the course of treatment. All but one patient, reporting nausea, completed his course of treatment. This patient was convalescing from hepatitis. One patient could tolerate only 100 mg t.i.d. but took a total of 2,400 mg with an apparent cure of his hookworm infection. The observations on drug side effects were complicated by a simultaneous outbreak of food poisoning, with vomiting and diarrhea, in some of the study group.

REFERENCES

Bueding, E., and Swartzwelder, C., 1957. Anthelmintics. *Pharmacol. Rev.*, **9:** 329–365.

Frye, W. W., Swartzwelder, C., Lampert, R., Abadie, S. H., and Carson, C. B., Jr., 1957. An effective trichuricide suitable for oral administration. *Amer. J. Trop. Med. and Hyg.*, **6:** 890–893.

Miller, J. H., Anthony, S. O., Swartzwelder, J. C., Frye, W. W., and Lampert, R., 1958. Treatment of enterobiasis with dithiazanine. *Am. J. Digest. Dis.*, **3:** 229–231.

Swartzwelder, J. C., Frye, W. W., Muhleisen, J. P., Miller, J. H., Lampert, R., Pena Chavarria, A., Abadie, S. H., Anthony, S. C., and Sappenfield, R. W., 1957. Dithiazanine, an effective broad-spectrum anthelmintic. *J.A.M.A.*, **165:** 2063–2067.

Swartzwelder, J. C., Muhleisen, J. P., Abadie, S. H., Frye, W. W., Jones, C. A., Robertson, P. E., and Hebert, J. F., 1958. Therapy of strongyloidiasis with dithiazanine. *A.M.A. Arch. Int. Med.*, **101:** 658–661.

THE ISOLATION AND IDENTIFICATION OF *TRYPANOSOMA CRUZI* FROM RACCOONS IN MARYLAND

BRYCE C. WALTON,[1] PRESTON M. BAUMAN,[2] LOUIS S. DIAMOND, AND CARLTON M. HERMAN

Department of Zoology, University of Maryland; Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C.; Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland; and Patuxent Research Refuge, Bureau of Sport Fisheries and Wildlife, U. S. Department of the Interior, Laurel, Maryland

During the course of a survey of filariasis in small mammals from the Patuxent Research Refuge, Laurel, Maryland, trypanosomes were observed in fresh blood preparations obtained from three juvenile raccoons (*Procyon lotor*) live-trapped in November, 1955. Attempts to isolate and identify this trypanosome were undertaken when Giemsa-stained preparations of the organisms demonstrated striking resemblance to *Trypanosoma cruzi* and when serum samples from these raccoons submitted to the Department of Serology, Walter Reed Army Institute of Research, proved to have a reactivity of high titer in a complement-fixation test (Chaffee *et al.*, 1956). The present report covers isolation and identification of this parasite. An abstract of these findings has been published (Walton *et al.*, 1956).

MATERIALS AND METHODS

Procedures for cultural isolation of trypanosomes from the larger mammals were the same as those employed by Diamond and Rubin (1958). Isolation from smaller mammals was accomplished by introduction of a few drops of cardiac blood directly into the culture medium. In the majority of isolations 1,000 units of potassium penicillin G + 0.01 gm of dihydrostreptomycin were added per ml medium. The medium employed was the STB medium of Diamond and Rubin (*loc. cit.*). All cultures were incubated at 23° C. A culture was considered negative if no growth occurred within 28 days. Subcultures were made at approximately 2-week intervals. The morphology of the trypanosomes was studied in thin smears of peripheral blood which were fixed by the method of Diamond and Herman (1954), and stained for 20 to 30 minutes in a Giemsa solution of pH 6.6 (Lillie, 1941). Tissue stages were studied in histological sections. Details of this technique are given by Diamond and Rubin (1958). Hegner's method (1921) of measuring trypanosomes was used. The image was projected at 2,500 diameters and measured with a pair of compasses rather than with a chartometer.

Infectivity was tested in five species of mammals in addition to raccoons; viz., young Swiss mice (*Mus musculus*), young laboratory-reared deer mice (*Peromyscus leucopus*), hairless suckling white rats (*Rattus rattus*), young laboratory-reared opossums (*Didelphis marsupialis*), and an adult female monkey (*Macaca irus*). The raccoons included 3 mature laboratory-raised, three mature wild-trapped, and two juvenile wild-caught animals. These raccoons and the monkey were not used until shown to be free from natural trypanosome infection upon repeated blood smear examinations and a 28-day hemoculture. Infectivity was also tested in four species of laboratory-reared triatomes known to be free of any trypanosomids.

Where possible, the diagnosis of the infection in all experimental animals was made by the examination of fresh blood in citrate-saline and stained smears. When trypanosomes were not found in the smears, inoculation of cardiac blood into STB medium or xenodiagnosis was used.

Serological tests, involving agglutination reactions, were conducted using antibodies induced in adult domestic rabbits, and living cultural trypanosomes as antigen. Control samples of blood were obtained in all instances prior to immunization. Two rabbits (207 and 208) were inoculated with the raccoon trypanosome, two (209 and 210) with *T. conorhini*, and two (211

[1] Present address: 406th Medical General Laboratory, APO 343, San Francisco, California.

[2] Present address: Parasitology Department, Research Division, Abbott Laboratories, North Chicago, Illinois.

and 212) with *T. cruzi* (Brazil strain). The inocula were prepared from 12 to 16-day cultures, the organisms being washed in 0.85% NaCl solution as in the preparation of antigen (*vide infra*), and the suspension standardized to contain about 7×10^6 trypanosomes per ml. Each animal received three 1-ml doses per week, the inocula being introduced via the marginal ear vein. Nine days after the last injection they were bled by cardiac puncture; the sera were separated aseptically and stored at −20° C.

Antigen for the tests was prepared from 12 to 16-day cultures. The overlay was centrifuged for 3 to 4 minutes at 700 G to remove debris. The supernate containing trypanosomes was then decanted, an equal volume of 0.85% NaCl solution added, and the organisms sedimented by centrifuging for 30 minutes at 1,000 G. After removing the supernate carefully with a capillary pipette, the packed trypanosomes were resuspended in salt solution sufficient to give a final count of approximately 7×10^6 organisms per ml. Sera to be tested were heated for 30 minutes at 56° C, and serial 2-fold dilutions, 1:10 to 1:20,480, prepared in 0.85% NaCl solution. The tests were performed on 2 x 3 inch glass slides with 12 paraffin rings of approximately 14 mm diameter. Aliquots (0.05 ml) of the serum dilutions were pipetted into separate rings, and 0.05 ml of antigen added. The slides were rotated mechanically for 5 minutes at 95 rpm, and then allowed to stand at room temperature. After 30 minutes the degree of agglutination was read microscopically at 100× magnification.

RESULTS

Two forms, a short, broad trypanosome (Fig. 1a) and an elongate, slender trypanosome (Fig. 1b), were observed in Giemsa-stained smears of peripheral blood from the raccoon. The former trypanosomes were present at all stages of infection; the latter were present in the early stages and gradually disappeared or became extremely rare as infection progressed. It was estimated that 25 per cent of the trypanosomes which were observed in the first few days after the parasites

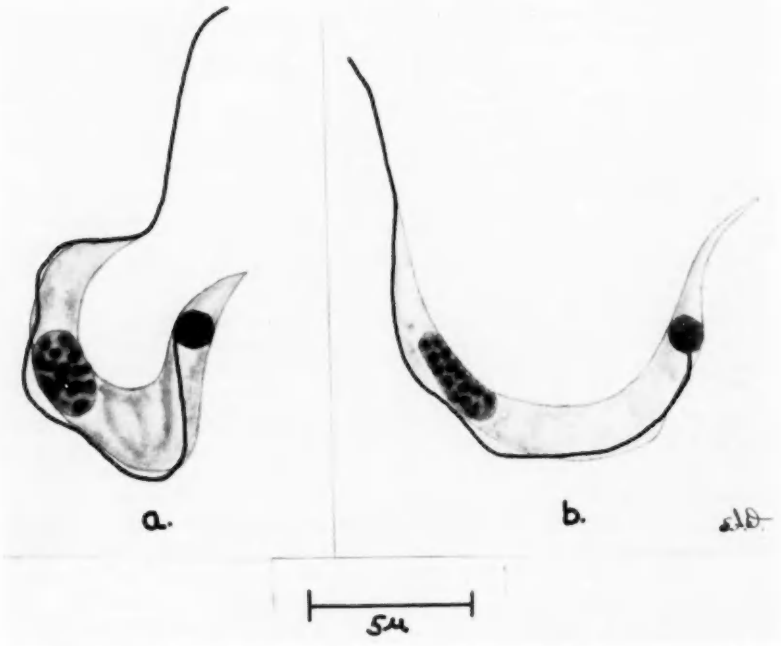


FIG. 1. Trypanosomes from peripheral blood of an experimentally infected raccoon (264): a, broad form; b, slender form. Drawn with aid of camera lucida, 5,000 diam.

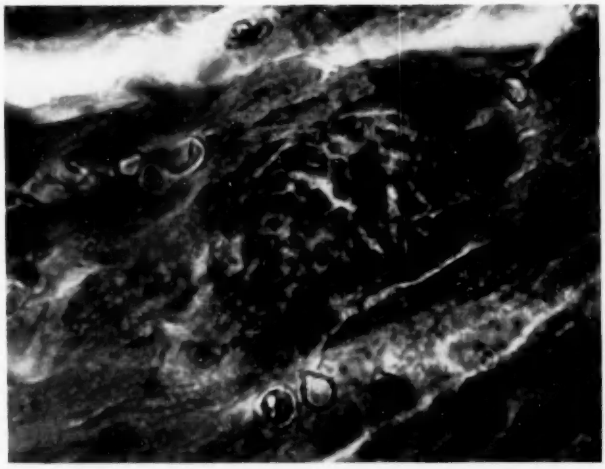


FIG. 2. Section of heart muscle from naturally infected raccoon (258) showing cluster of typical leishmaniform bodies, ×720.

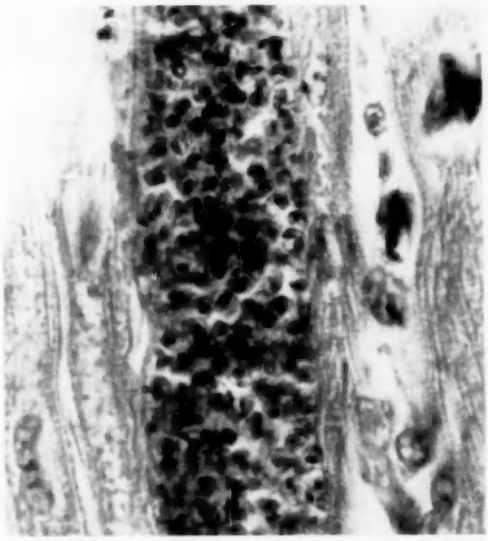


FIG. 3. Cluster of leishmaniform bodies in section of heart muscle from experimentally infected racoon(300), × 1620.

made their appearance in peripheral blood, were of the slender type.

Measurements are presented of the first 50 trypanosomes observed in a peripheral blood film obtained 13 days after inoculation of a raccoon (264) with culture material. The mean, standard error, standard deviation, and coefficient of variation of each of ten measurements made per trypanosome are as follows: Total length, 22.6 μ ± 1.7, ±1.21, 5.4; width, 1.5 μ ± .03, ±0.21, 13.7; posterior extremity to kinetoplast, 2.1 μ ± .07, ±0.47, 22.0; kinetoplast to center of nucleus,

TABLE 1

Agglutination Tests of Six Non-Immunized Rabbit Sera Against Trypanosoma cruzi (*Brazil Strain*), T. conorhini, *and the Raccoon Trypanosome*

Antigen	Serum Dilution						
	1:10	1:20	1:40	1:80	1:160	1:320	1:640
Rabbit #207							
Brazil Strain	+	+	+	−	−	−	−
T. conorhini	+	+	+	+	−	−	−
Raccoon Trypanosome	+	+	+	+	+	−	−
Rabbit #208							
Brazil Strain	+	+	+	−	−	−	−
T. conorhini	+	+	+	+	−	−	−
Raccoon Trypanosome	+	+	+	+	+	−	−
Rabbit #209							
Brazil Strain	+	+	−	−	−	−	−
T. conorhini	+	+	+	−	−	−	−
Raccoon Trypanosome	+	+	+	+	+	+	−
Rabbit #210							
Brazil Strain	+	+	+	−	−	−	−
T. conorhini	+	+	+	−	−	−	−
Raccoon Trypanosome	+	+	+	+	−	−	−
Rabbit #211							
Brazil Strain	+	+	+	+	+	−	−
T. conorhini	+	+	+	+	−	−	−
Raccoon Trypanosome	+	+	+	+	+	−	−
Rabbit #212							
Brazil Strain	+	+	+	−	−	−	−
T. conorhini	+	+	+	+	−	−	−
Raccoon Trypanosome	+	+	+	+	+	−	−

7.8 μ ± .10, ±0.69, 8.8; center of nucleus to anterior extremity, 5.7 μ ± .09, ±0.64, 11.3; flagellum, 7.0 μ ± .13, ±0.89, 12.8; nuclear index[3], 1.8 μ ± .03, ±0.23, 13.0; kinetoplast, 1.1 μ ± .02, ±0.12, 11.0; nuclear length, 2.5 μ ± .04, ±0.26, 10.3; nuclear width, 1.5 μ ± 0.3, ±0.20, 13.3.

[3] Nuclear index is the distance from posterior extremity to center of nucleus divided by distance from center of nucleus to anterior extremity of a trypanosome.

Typical leishmaniform stages (Figs. 2 and 3) were found in cardiac muscle cells in one naturally infected (258) and one experimentally infected raccoon (300). Similar stages were found in the heart of an experimentally infected mouse.

The organisms grew readily on culture media. Leishmaniform, crithidiform, and trypanoform stages were observed. Upon primary isolation, the metacyclic trypanoform stages become abundant in from 10 to 12 days, but after several subcultures this condition was not attained until 4 to 6 weeks following inoculation of the medium. Culturing of the trypanosome seems to have little effect on its infectiousness; a culture isolated from a natural infection on 28 November 1955 was still infective for raccoons 284 days later, at which time it was in the 20th subculture.

In raccoons, the three types of inocula (blood, culture, and triatomids) produced nonfatal infection with eventual disappearance of organisms from peripheral blood, although there was considerable variation during the period when they were present. In most cases trypanosomes remained quite sparse, usually with only one or two organisms found in a one-drop preparation. However, one raccoon (264), inoculated with cultural forms, exhibited small numbers of trypanosomes from the 5th day until the 13th day after infection when it then showed numbers equal to, or greater than, those first observed in the natural infections, with an organism to every one or two high-power fields. This level persisted until the 11th week when it rapidly began to fall and trypanosomes could no longer be demonstrated in blood smears after the 14th week. However, cardiac blood taken from both animals, 21 days after the last positive smear, gave positive cultures. No clinical signs were detected.

The two immature wild raccoons inoculated with culture material 284 days after original isolation (20th subculture) showed peripheral forms on the fifth and eighth days, respectively, after inoculation. One raccoon (300) died after cardiac bleeding on the 12th day. Microscopic examination of cardiac tissue revealed the presence of numerous large lesions containing leishmaniform stages (Fig. 3). The survivor was still exhibiting trypanosomes in peripheral blood smears 8 weeks after inoculation.

Young mice, inoculated with cultural forms,

TABLE 2

Cross Agglutination Tests of Rabbit Antisera Against Trypanosoma cruzi (*Brazil Strain*), T. conorhini, *and the Raccoon Trypanosome*

Antigen	Serum Dilution											
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240	1:20,480
Rabbit #207 Raccoon-trypanosome Antiserum												
Brazil Strain	+	+	+	+	+	+	+	+	−	−	−	−
T. conorhini	+	+	+	+	+	−	−	−	−	−	−	−
Raccoon Trypanosome	+	+	+	+	+	+	+	+	+	+	−	−
Rabbit #208 Raccoon-trypanosome Antiserum												
Brazil Strain	+	+	+	+	+	+	+	+	+	−	−	−
T. conorhini	+	+	+	+	+	+	+	−	−	−	−	−
Raccoon Trypanosome	+	+	+	+	+	+	+	+	+	+	−	−
Rabbit #209 *T. conorhini* Antiserum												
Brazil Strain	+	+	−	−	−	−	−	−	−	−	−	−
T. conorhini	+	+	+	+	+	+	+	−	−	−	−	−
Raccoon Trypanosome	+	+	+	+	−	−	−	−	−	−	−	−
Rabbit #210 *T. conorhini* Antiserum												
Brazil Strain	+	+	+	−	−	−	−	−	−	−	−	−
T. conorhini	+	+	+	+	+	+	−	−	−	−	−	−
Raccoon Trypanosome	+	+	+	+	−	−	−	−	−	−	−	−
Rabbit #211 *T. cruzi* (Brazil Strain) Antiserum												
Brazil Strain	+	+	+	+	+	+	+	+	−	−	−	−
T. conorhini	+	+	+	−	−	−	−	−	−	−	−	−
Raccoon Trypanosome	+	+	+	+	+	+	−	−	−	−	−	−
Rabbit #212 *T. cruzi* (Brazil Strain) Antiserum												
Brazil Strain	+	+	+	+	+	+	+	+	−	−	−	−
T. conorhini	+	+	+	+	−	−	−	−	−	−	−	−
Raccoon Trypanosome	+	+	+	+	+	+	+	−	−	−	−	−

developed nonfatal infections, usually with very low parasitemias that persisted in the blood for 2 to 8 days. One animal developed large numbers, with one or two to every high-power field. Inoculation with infective raccoon blood was less effective, and produced infections in only six of eight suckling mice and one of five 8 to 10-gm mice. Positive cultures from inocula of heart blood, minced liver, spleen, and kidney demonstrated persistence of the infection beyond the time organisms could be found in the blood.

Three of five mice, inoculated with organisms from pooled gut contents of four *Triatoma protracta* which were sacrificed 9 days after infective feeding, developed nonfatal infections, although the initial appearance of trypanosomes in the blood was delayed until the 15th day.

All five deer mice inoculated appeared refractory to infection as no trypanosomes were seen in the blood through 21 days and specimens of *Rhodnius prolixus* fed on the mice at the end of this period showed no flagellates in the gut on dissection 32 days after feeding.

Young laboratory rats, inoculated with cultural forms, developed infections similar to those produced in mice except that the trypanosomes tended to be present in greater numbers and persisted up to 9 days after their initial appearance.

Of three opossums inoculated with cultural

forms of the raccoon trypanosomes, only one (202) exhibited trypanosomes in the blood, and these appeared only on the 24th day. Opossum 201 showed no organisms up to its death on the 15th day. A post-mortem heart-blood-sample culture developed bacterial contamination and was discarded. Animal 203 exhibited no trypanosomes through 30 days, but cultures of heart blood taken on the 30th day proved positive.

Two of three opossums inoculated with the Brazil strain exhibited blood forms. Opossum 205 had trypanosomes on the 20th and on the 23rd day through the 28th day; 206 showed trypanosomes on the 21st through the 28th day; while 204 never exhibited organisms through 30 days and was negative on xenodiagnosis at that time.

In the *Macaca irus*, trypanosomes appeared in the blood on the 17th day after inoculation and increased in numbers to the 21st day. The level varied irregularly for approximately 2 weeks, then became relatively constant at a low level, and was demonstrable only in thick-smear preparations. Organisms persisted in the peripheral blood until the 9th week.

Adults and older nymphs of *Triatoma protracta* showed many crithidial forms in the mid-gut and few in the hind-gut when sacrificed on the third day after the infective feeding. No metacyclic forms were seen in routine stained smears of gut contents of bugs sacrificed on the 9th day, although this inoculum produced infections in mice. Metacyclic forms were seen in smears of gut contents 30 days and 49 days after infection. Infection in the triatomes was evidently confined to the mid- and hind-gut, as no organisms were found in hemolymph collected at the coxal joint, and none in the anterior portion of the gut or in the salivary glands up to 49 days after infection. One feeding produced an infection in all of the 13 bugs of this species examined. *Triatoma phyllosoma*, *T. infestans*, and *Rhodnius prolixus* likewise became infected, and crithidial and trypanosomal forms were found in the feces when examined 21 days after feeding.

Preimmunization sera from the six rabbits agglutinated all three trypanosomes. As indicated in Table 1, the titers, generally, were highest with the raccoon-trypanosome antigen, intermediate with *T. conorhini*, and lowest with *T. cruzi* (Brazil strain). Immunization with any of the three parasites increased predominantly the titer of homologous antibody (Table 2). It is noteworthy, however, that the raccoon trypanosome and *T. cruzi* each induced a high degree of cross reactivity for the other, while causing relatively little increase in agglutinins for *T. conorhini*. Immunization with *T. conorhini*, conversely, had no detectable influence on reactivity with the raccoon trypanosome or *T. cruzi*.

DISCUSSION

The raccoon trypanosome is morphologically indistinguishable from *T. cruzi*. The occurrence of both slender and broad trypanosomes in our material is in keeping with findings of other investigators (Chagas, 1909; Brumpt, 1912; Dias, 1934; Wood, 1951; and Pizzi, 1953). The developmental cycle in the vertebrate host includes typical leishmaniform stages within cardiac muscle fibers, a characteristic of *T. cruzi*.

The ability of the raccoon strain to infect a wide range of mammals is demonstrated in this paper by the success obtained in transferring it to mice, rats, opossum, and monkeys. Concurrent studies, in which domestic cattle, goats, sheep, and pigs were infected (Diamond and Rubin, 1956) with this same strain, further emphasize this point. Ability of the raccoon trypanosome to infect monkeys is considered to be of particular significance, since this can be considered strong presumptive evidence that it could produce an infection in man.

The stages present and the rate and amount of growth in culture of the raccoon trypanosome were the same as those of *T. cruzi* (Brazil strain).

Cross agglutination studies were conducted utilizing the raccoon trypanosome, *T. cruzi* (Brazil strain), and *T. conorhini*. This last-named species is a parasite of rodents transmitted by triatomids. Morphologically it can be placed in Group A of Hoare's system (1957) of classification of mammalian trypanosomes which includes *T. cruzi*. Further, von Brand and Johnson (1947), on the basis of physiological studies of mammalian trypanosomes, place *T. conorhini* in the same group. Since a strain of *T. conorhini*, as well as *T. cruzi*, was available to us, this material was used for comparison.

Antigenic relations, some indications of which were provided by tests of the preimmunization sera, were more clearly brought out in tests of the antisera. The degree of the cross-agglutination reactions elicited by the raccoon trypanosome and *T. cruzi* indicated a closer antigenic relation between each other than between either of these and *T. conorhini*.

Since the antigenic differences between the raccoon trypanosome and *T. cruzi* were fewer than those exhibited between *T. cruzi* and *T. conorhini*, the differences may be interpreted taxonomically as being of infraspecific level, because they are smaller than those exhibited between the two closely related recognized species within the *lewisi* group. Some infraspecific antigenic distinctions have been demonstrated between several strains of *T. cruzi* in mouse antisera (Hauschka *et al.*, 1950), and it has been shown that *T. brucei* and *T. hippicum* antisera from rabbits agglutinate *T. cruzi* only in extremely low dilutions in contrast to agglutination in extremely high dilutions of *T. cruzi* antiserum (Packchanian, 1940). While these data are not directly comparable to the results reported here, they do indicate an additional basis for the interpretation of the antigenic differences between the raccoon trypanosome and the Brazil strain of *T. cruzi* as being at a strain-difference level and not at the level of distinct species.

The present study has shown that the Maryland raccoon trypanosome cannot be differentiated from *Trypanosoma cruzi* (Chagas, 1909) in any important regard, and on the basis of the criteria employed it must be regarded as conspecific with the etiological agent of human American trypanosomiasis.

Occurrence of this parasite in an area so far removed from previously known endemic areas is of particular interest in light of two recent reports of the first indigenous cases of human trypanosomiasis in the United States (Woody and Woody 1955; Nat'l. Off. Vital Stat., 1955). Previous reports of natural *T. cruzi* infections of mammals in the United States are reviewed by Craig and Faust (1951) and Wood (1952), and include the opossum, wood rats, deer mice, house mouse, bats, and armadillo. These records are all from Southwestern United States from localities in Texas, New Mexico, Arizona, and California. Mammalian infections are generally considered to be associated with high infection rates in the triatome vectors which live in close association with the infected mammalian population. The Maryland locality is within the range reported for *Triatoma sanguisuga* and *T. lecticularius*, (Usinger, 1944), both species being known vectors of Chagas' disease. However, upon the basis of numbers of specimens in collections made from this area, current information indicates that these insects may be quite rare in the region (Sailer, 1956, personal communication). Since this study was initiated, trypanosomes have been diagnosed from 5 of 400 raccoons examined but no evidence of infection in other wild animals, or its means of transmission in this area, has yet been obtained. Habermann *et al.*, (1958) reported tissue stages of *T. cruzi* from a raccoon trapped in Maryland and Brooke *et al.*, (1957) isolated *T. cruzi*-like organisms from skunk, raccoon, and opossum in Georgia.

ACKNOWLEDGMENTS

Special thanks are extended to Donald B. McMullen, Chief, Department of Medical Zoology, WRAIR, for expediting the initiation of these studies. The authors wish to express their thanks to the following individuals for their generous contributions which materially aided this investigation: Leonard Llewellyn, Patuxent Research Refuge, for providing the wild animals used and for advice and assistance in handling them; J. I. Bruce, R. L. Bray, and A. E. Dennis, WRAIR, for technical assistance; H. S. Fuller, WRAIR, and P. A. Woke, NIH, for providing triatomes: Eleanor Tobie, NIH, for cultures of *Trypanosoma conorhini*; J. F. Kent, J. C. Burke, and E. H. Fife, WRAIR, for cultures of Brazil strain of *T. cruzi* and for advice and assistance on serological tests; Betty Merrill, WRAIR, and Donald Thompson, Animal Parasite Laboratory, ADP, ARS, for preparing tissue slides; and M. O. Cheskis and SP2 Sally L. Craig for photomicrographs.

A portion of this paper is included in a thesis submitted by the senior author to the Department of Zoology, University of Maryland, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. He wishes to express grateful acknowledgment for the help and guidance of Professor G. W. Wharton.

SUMMARY

Five raccoons trapped at Patuxent Research Refuge, Laurel, Maryland, were found to have trypanosomes in the blood which were morphologically indistinguishable from *Trypanosoma cruzi* on stained smears. The organism grew well in culture. It developed and reproduced in *Triatoma protracta*, *T. infestans*, *T. phyllosoma*, and *Rhodnius prolixus*. Experimental infections were produced in raccoons, opossums, mice, rats, and monkeys by inoculation of blood, culture, and triatome forms. Typical leishmaniform bodies

were found in tissue sections of cardiac muscle fibers from naturally and experimentally infected animals. Cross agglutinations carried out with living cultural forms and rabbit antisera demonstrated a close antigenic relationship between the raccoon trypanosome and *T. cruzi* (Brazil strain). On the basis of (1) morphology, (2) presence of leishmaniform tissue stages, (3) development in triatomes, (4) infectivity to a variety of mammals, (5) culture characteristics, and (6) cross reactions in serological tests, this parasite is considered conspecific with *Trypanosoma cruzi* (Chagas, 1909) the causative agent of American human trypanosomiasis.

REFERENCES

VON BRAND, T., AND JOHNSON, ELEANOR T., 1947. A comparative study of the effect of cyanide on the respiration of some Trypanosomidae. *J. Cell. & Comp. Phys.*, **29:** 33–49.

BROOKE, M. M., NORMAN, L., ALLAIN, A., AND GORMAN, G. W., 1957. Isolation of *Trypanosoma cruzi*-like organisms from wild animals collected in Georgia. *J. Parasitol.*, **43** (suppl.): 15.

BRUMPT, E., 1912. Le *Trypanosoma cruzi* évolue chez *Conorhinus megistus*, *Cimex lectularius*, *Cimex boueti* et *Ornithodorus moubata*. Cycle évolutif de ce parasite. *Bull Soc. Pathol Exotique*, **5:** 360–367.

CHAFFEE, E. F., FIFE, E. H., JR., AND KENT, J. F., 1956. Diagnosis of *Trypanosoma cruzi* infection by complement fixation. *Am. J. Trop. Med. & Hyg.*, **5:** 763–771.

CHAGAS, C., 1909. Novo tripanzomiaze humana. Estudos sobre a morfolojia e o ciclo evolutivo do *Schizotrypanum cruzi* n. gen., n. sp., ajente etiolojico de nova entidade morbida do homen. *Mem. Inst. Oswaldo Cruz*, **1:** 159–218.

CRAIG, C. F., AND FAUST, E. C., 1951. *Clinical Parasitology*, 5th ed. Lea & Febiger, Philadelphia, Pa. 1032 pp.

DIAMOND, L. S., AND HERMAN, C. M., 1954. Incidence of trypanosomes in the Canada goose as revealed by bone marrow culture. *J. Parasitol.*, **40:** 195–202.

DIAMOND, L. S., AND RUBIN, R., 1956. Susceptibility of domestic animals to infection with *Trypanosoma cruzi* from the raccoon. *J. Parasitol.*, **42** (suppl.): 21.

DIAMOND, L. S., AND RUBIN, R., 1958. Experimental infection of certain farm mammals with a North American strain of *Trypanosoma cruzi* from the raccoon. *Exptl. Parasitol.*, **7:** 383–390.

DIAS, E., 1934. Estudo sobre o *Schizotrypanum cruzi*. *Mem. Inst. Oswaldo Cruz*, **28:** 1–110.

HABERMANN, R. T., HERMAN, C. M., AND WILLIAMS, F. P., 1958. Distemper in raccoons and foxes suspected of having rabies. *J. Am. Vet. M. A.*, **132:** 31–35.

HAUSCHKA, T. S., GOODWIN, M. B., PALMQUIST, J., AND BROWN, E., 1950. Immunological relationships of seven strains of *Trypanosoma cruzi*. *Am. J. Trop. Med.*, **30:** 1–16.

HEGNER, R. W., 1921. Measurements of *Trypanosoma diemyctyli* from different hosts and their relation to specific identification, heredity and environment. *J. Parasitol.*, **7:** 105–113.

HOARE, C. A., 1957. The classification of trypanosomes of veterinary and medical importance. *Vet. Revs. & Annotations*, **3:** 1–13.

LILLIE, R. D., 1941. Romanowsky staining with buffered solution, III. Extension of the method to Romanowsky stains in general. *Stain Tech.*, **16:** 1–6.

National Office of Vital Statistics, 1955. Communicable Diseases Summary for week ending Dec. 10, 1955. U. S. Dept. Health Education & Welfare, Washington 25, D. C.

PACKCHANIAN, A., 1940. Experimental production of agglutinins for *Trypanosoma cruzi*. *Pub. Health Rept.*, **55:** 2116–2124.

PIZZI, T., 1953. Sobre el problema de las formas delgadas de *Trypanosoma cruzi*. *Bol. Inf. Parasitol. Chilenas*, **8:** 26–30.

USINGER, R. L., 1944. The Triatominae of North and Central America and the West Indies and their Public Health significance. *Pub. Health Bull.* no. 288, 81 pp.

WALTON, B. C., BAUMAN, P. M., DIAMOND, L. S., AND HERMAN, C. M., 1956. *Trypanosoma cruzi* in raccoons from Maryland. *J. Parasitol.*, **42** (suppl.): 20.

WOOD, S. F., 1951. Development of Arizona *Trypanosoma cruzi* in mouse muscle. *Am. J. Trop. Med.*, **31:** 1–11.

WOOD, S. F., 1952. Mammal blood parasite records from Southwestern United States and Mexico. *J. Parasitol.*, **38:** 85–86.

WOODY, N. C., AND WOODY, H. B., 1955. American trypanosomiasis (Chagas' disease)—first indigenous case in the United States. *J. A. M. A.*, **159:** 676–677.

STUDIES ON THE USE OF CORTISONE AND ACTH IN TRICHINOSIS[1]

RICHARD A. LORD[2]

Department of Microbiology, Yale University School of Medicine, New Haven, Connecticut

INTRODUCTION

The incidence of trichinosis in the general population of the United States is thought to be about 25 per cent, but may be as high as 35 per cent in some regions. Most cases, however, are subclinical and go unrecognized. In those cases which come to medical attention the mortality rate is about 5 per cent. No specific therapy is known, and the treatment is symptomatic (Gould, 1945). In recent years clinical trials have shown all the anthelminthic drugs to be ineffective in the intramuscular phase of this disease.

Since 1951, clinical studies on the use of ACTH and cortisone have yielded data showing that these drugs are valuable in the treatment of trichinosis (Buyella *et al.*, 1953; Davis and Most, 1951; Faiguenbaum, 1952; Rosen, 1952; Rothenberg, 1951; Sadusk, 1954; Solomon and Seligman, 1952). Significant relief of symptoms has been reported: decrease in fever and eosinophilia, increased muscular strength, and a lessening of pain and fatigue. Studies on experimental trichinosis by other workers have given less clear-cut results (Luongo *et al.*, 1951; Pollay *et al.*, 1954; Stoner and Godwin, 1954).

On the basis of these findings, this investigation was designed to test the effects of ACTH and cortisone on experimentally-produced trichinosis in rats with regard to the change in weight of the animals during the course of the disease, the occurrence of eosinophilia, the survival time, and the tissue response of the host to the invading organism. It was hoped to show if either of these drugs produced objective improvement as well as the symptomatic relief which had been reported, and if the disease process, in this case the multiplication and encystment of the larvae, was affected by the treatment.

METHODS AND MATERIALS

Fifty-nine 5-week-old male albino rats weighing approximately 200 gm were used in the experiment. These animals were divided into groups of four and, after anesthetization with pentobarbital intraperitoneally, were infected with suspensions of pepsin-digested rat or guinea pig skeletal muscle containing 180, 1,000, 2,500, and 5,000 viable larvae, respectively. The inoculum was administered from a 10-ml syringe through a polyethylene catheter passed down the esophagus and into the stomach. A volume of saline was used sufficient to make a total volume of 10 ml in each case.

On the 6th day after inoculation treatment was begun with the following dosages: ACTH: 1 international unit (IU) per day in 0.1 ml of solution containing ten IU per ml, given intramuscularly (IM). This was given every day for 39 days. Cortisone: 5 mg per day for 10 days, given as 0.2 ml of solution containing 25 mg per ml, given IM; then 2.5 mg per day for 2 days; then 1.0 mg per day for 14 days; thereafter, 0.5 mg per day for 13 days. Hence a total of 75.5 mg cortisone per animal was given over a 39-day

TABLE 1

Each line refers to a group of four animals

Larvae in infective dose	ACTH or Cortisone	Larvae in 50 medium-power fields	Survival
			(*days*)
180	—	5–26	33–8*
180	ACTH	3–20	8
180	C	14–45	24–8
1,000	—	18–29	60–8
1,000	ACTH	8–18	8
1,000	C	0†–170	7–58
2,500	—	7	47–8
2,500	ACTH	8–47	8
2,500	C	0†–208	16–55
5,000	—	46–378	33–8
5,000	ACTH	25–446	34–8
5,000	C	90–190	17–23

* Animal survived the experiment and was sacrificed on the 87th day.

† Death occurred during the intestinal phase of the disease.

[1] This work was supported in part by the James Hudson Brown Memorial Fund.

[2] Present address: Department of Pediatrics, Yale University School of Medicine.

period while, during the same period, 39 IU ACTH were given.

Specimens of muscle tissue from those animals dying during the course of the experiment and from those killed when the experiment was terminated 87 days after inoculation were taken and preserved in 10 per cent formalin. Sections were made from gastrocnemius muscle and stained with hematoxylin and eosin. During the first 45 days of the experiment the animals were weighed, temperatures taken, and eosinophil counts done twice weekly. Thereafter, a weekly weighing was the only determination made.

A preparation of desiccated ACTH (Armour) assayed at 1.75 IU per mg was used. Cortisone was used as cortisone acetate (Merck).

RESULTS

Weight; ACTH. The gain in weight over a 6-week period in animals of the same age and initial weight was the same for the untreated, uninfected rats as for those receiving 1 IU ACTH per day over this period. Hence, ACTH in this dosage had no effect on the rate of growth of normal animals, and any curve of growth of animals receiving ACTH might be taken as a curve of normal growth.

In animals infected with 180 larvae, those

FIG. 1. Section from animal No. 13 infected with 1,000 larvae and receiving no medication. The animal survived and was sacrificed and autopsied 87 days after infection. Larvae are well encapsulated and cellular infiltration is present around them.

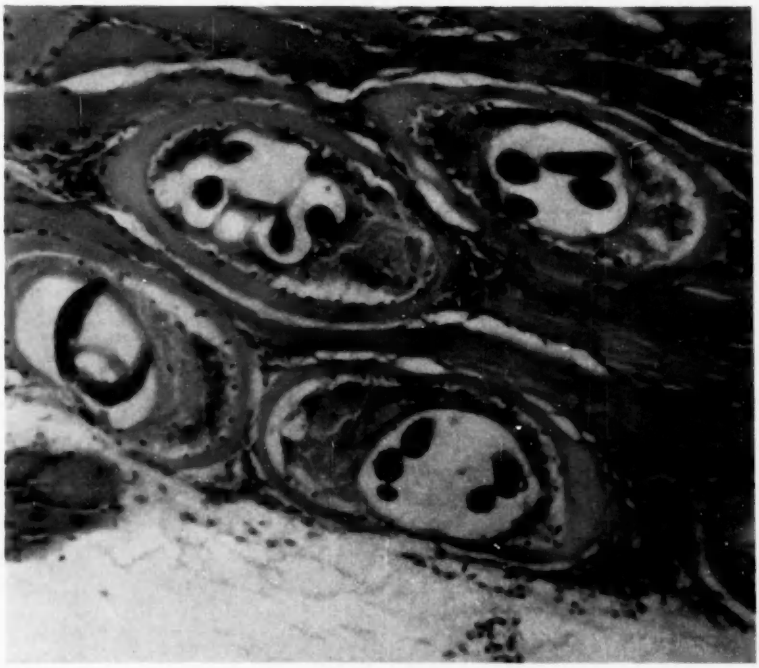


FIG. 2. Section from animal No. 20 infected with 1,000 larvae and treated with ACTH. Autopsy 87 days after infection. Encapsulation of the larvae and cellular response appear the same as in Figure 1.

treated with ACTH gained weight in a manner indistinguishable from that of controls receiving ACTH only, while controls which were infected with the same number of larvae, but were untreated, lost weight. A 32 per cent difference in weight gain after 60 days was noted between treated and untreated groups. With infection at this low level ACTH offset completely the loss in weight caused by the disease.

In animals infected with 1,000 larvae those given ACTH fared only slightly worse than uninfected controls; the difference being 6 per cent at 60 days. A 24 per cent difference in weight change was observed between the infected and treated, and the infected but untreated, at 60 days. Hence, as the size of the infecting dose of larvae was increased the dose of ACTH was less capable of offsetting the loss in weight due to disease, but could reduce weight loss by about 80 per cent in untreated animals: untreated animals lost 30 per cent, treated, 6 per cent; 24/30 = 80 per cent.

In animals infected with 2,500 larvae, those treated with ACTH were 5 per cent below their uninfected controls in weight; however, these two weight curves plotted against time were more widely separated throughout their course than similar curves of the 1,000-larvae group. The difference between treated and untreated groups was 36 per cent. Decrease in gain of weight was seen as the infecting dose was increased, but excellent protection against such loss was still afforded by ACTH.

In animals infected with 5,000 larvae, those treated with ACTH were 36 per cent below uninfected controls in weight gain. There was no significant difference between treated and untreated infected animals. In heavy infections ACTH provided no protection against weight loss.

With increasing doses of larvae all curves of weight gain were depressed in infected animals.

Weight; Cortisone. At dosages of 180 and 1,000 larvae, animals treated with cortisone and treated, uninfected controls were nearly the same.

The infected controls showed slightly less weight loss. As the number of infecting larvae was increased, the effect of the disease process as well as the toxicity of cortisone came into evidence. As the infective dose was further increased, the disease process and cortisone ceased to exert their effects independently, and cortisone augmented the disease process or vice versa.

Survival and Mortality. ACTH decreased mortality even at the highest level of infection. Cortisone toxicity alone accounted for a 50 per cent mortality, and had no protective effect for the host. The results suggested that the toxicity of cortisone and the effect of the infection were independent of each other with respect to mortality.

ACTH was very effective in increasing survival time. All animals except those with the heaviest infection survived. Of the cortisone-treated animals, those infected with 180 and 1,000 larvae showed only a decrease in survival time due to cortisone alone, *i.e.*, the decrease in survival time was caused by cortisone and was not augmented by the disease. As the infecting dose was increased, the survival time was decreased by more than the sum of cortisone-toxicity-plus-disease, suggesting that these two factors were no longer operating independently.

Between the 60th and 87th day after infection, all groups gained weight at approximately the same rate, indicating that the disease had run its course by the 60th day and that the drugs no longer had any effect.

Eosinophilia. The eosinophil response in untreated, infected animals reached a peak of 10 to 12 per cent between 10 to 30 days after infection and returned gradually to normal (0 to 2%). Eosinophilia was slightly reduced by ACTH, and the appearance of the peak value was retarded by about 5 days. Eosinophilia was suppressed by cortisone in all cases.

Tissue Response. This is summarized in Table

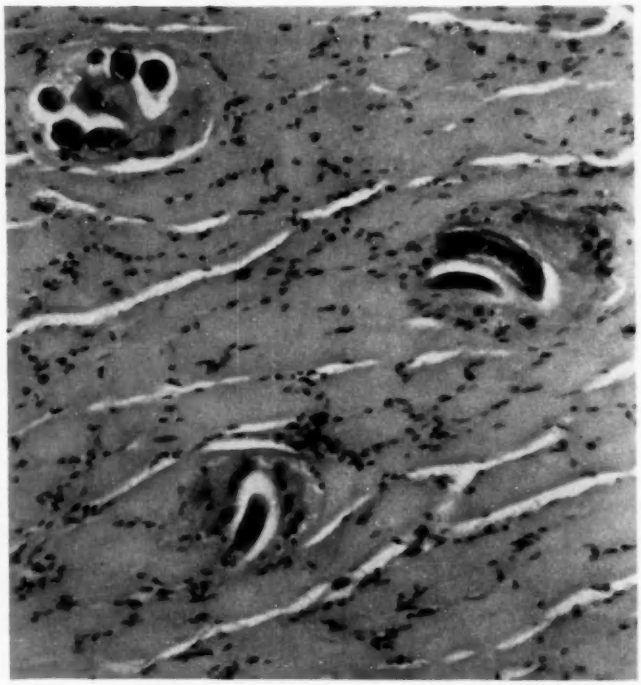


FIG. 3. Section from animal No. 23 infected with 1,000 larvae and treated with cortisone. The animal survived and was sacrificed and autopsied 87 days after infection. No encapsulation of the larvae is seen, and there is no cellular infiltration.

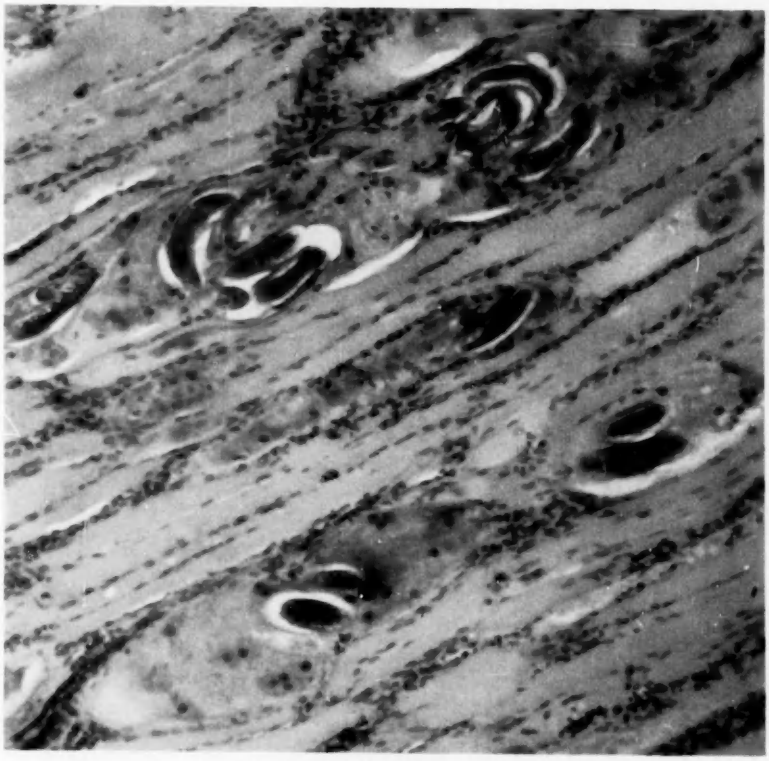


FIG. 4. Section from animal No. 40 infected with 5,000 larvae and receiving no medication. The animal died 46 days after infection. Many larvae are seen, there is slight encapsulation, and a pronounced cellular infiltration is present.

4 and illustrated in the photomicrographs. Fifty consecutive, medium-power, fields were counted on each slide. In animals treated with ACTH an inflammatory response consisting of polymorphonuclear leukocytes, encapsulation of larvae, and eosinophils was irregularly evident throughout the sections, except in those from animals receiving the heaviest larval infections and in those treated with cortisone, in which all three were absent. There were fewer larvae in the ACTH-treated groups than in either the untreated or cortisone-treated groups when the infecting dose was small. However, as the infection was increased there was no essential difference between groups in this respect. At lower levels of infection the groups treated with cortisone showed 2.5 to 10 times the number of larvae seen in untreated animals. It may be that one of the effects of cortisone in abolishing the tissue response in the intestinal mucosa was to permit a greater number of larvae to penetrate at this site and enter the circulation (Coker, 1955, 1956 a, b, c).

These observations are consistent with the findings in regard to weight, survival time, and mortality, *i.e.*, ACTH was beneficial to the host whereas cortisone was deleterious. It may be that cortisone, by interfering with encystment, exposed the host tissues to toxic products from the larvae for a longer time. Only one infected animal died while receiving ACTH. Larval encapsulation in its tissues was not different from that in similar, untreated animals.

DISCUSSION

The results of these experiments support the conclusion that ACTH therapy in trichinosis

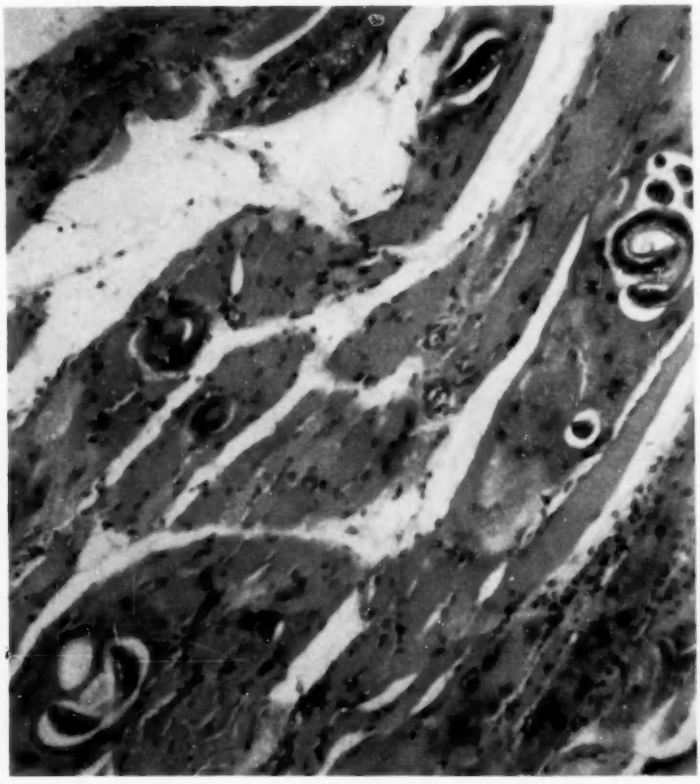


FIG. 5. Section from animal No. 45 infected with 5,000 larvae and treated with cortisone. The anima survived 23 days after infection. Numerous larvae are seen. There appears to be no encapsulation, and no cellular infiltration is present.

minimizes the loss of weight, decreases mortality, prolongs survival time, slightly reduces eosinophilia, and leaves the tissue response unaltered. In contrast, cortisone therapy seems to have no beneficial effect and, in the large doses used, actually was quite toxic. Whether it affects the response of muscle tissue to infection can not be decided from this work since it would have been necessary to sacrifice an untreated, infected control animal at the time each cortisone-treated animal died. However, the results of Coker's work (1955, 1956, a, b, c) make it appear likely that it does. The evidence suggests that more larvae were able to penetrate the intestinal mucosa in those animals receiving cortisone. This was probably another factor in its deleterious action.

Since ACTH is believed to act only by stimulating the adrenal cortex to secrete certain of its hormones, it was surprising to find that its action should be so different from that of cortisone. With few exceptions either of these drugs has been thought to be capable of replacing the other in therapy. One explanation for the differences noted may be that the dose of cortisone was simply too large and that qualitatively different results would have been obtained had the dose been reduced. Another explanation is that ACTH, in stimulating the adrenal cortex, caused hormones which act in a manner different from cortisone to be secreted, and that these were more beneficial to the host in its defense against parasitic infection than was cortisone. Large doses of cortisone, by decreasing ACTH levels,

would indirectly decrease the levels of such hormones. The results of these experiments do not provide data for evaluating these hypotheses.

REFERENCES

BUYELLA, P. A., *et al.*, 1953. Trichinose et ACTH, *Gaz. Méd. France*, **60:** 1171–1172.

CHUTE, R. M., 1956. The dual antibody response to experimental trichinosis. *Proc. Helminthol. Soc. Washington*, **23:** 49–58.

COKER, C. M., 1955. Effects of cortisone on *T. spiralis* infection in non-immunized mice. *J. Parasitol.*, **5:** 498–504.

COKER, C. M., 1956a. Some effects of cortisone in mice with acquired immunity to *Trichinella spiralis*. *J. Infect. Dis.*, **98:** 39–44.

COKER, C. M., 1956b. Cellular factors in acquired immunity to *T. spiralis* as indicated by cortisone treatment of mice. *J. Infect. Dis.*, **98:** 189–197.

COKER, C. M., 1956c. Effects of cortisone on cellular inflammation in the musculature of mice given one infection with *Trichinella spiralis*. *J. Parasitol.*, **42:** 479–484.

DAVIS, W. M., AND MOST, A., 1951. Trichinosis, case report with observations on the effect of ACTH. *Am. J. Med.*, **11:** 639.

FAIGUENBAUM, J. A., 1952. Triquinosis y cortisone, *Bol. Inform. Paras. Chil.*, **8:** 9–11.

GOULD, S. E., 1945. *Trichinosis*, Springfield, Illinois. Charles C Thomas.

LUONGO, M. A., REID, D. H., AND WEISS, W. W., 1951. The effect of ACTH in trichinosis, a clinical and experimental study. *New England J. Med.*, **245:** 757.

POLLAY, M., WEIN, B., AND HARTMANN, H. A., 1954. Effect of ACTH upon artificially induced trichinosis in rats with special reference to eosinophilia. *Proc. Soc. Exper. Biol. & Med. New York*, **86:** 577–580.

ROSEN, E., 1952. Cortisone treatment of trichinosis. *Am. J. M. Sc.*, **223:** 16–19.

ROTHENBERG, F., 1951. Treatment of Trichinosis with Cortisone. *J. Med. Soc. New Jersey*, **48:** 517.

SADUSK, J. F., 1954. Cortisone in treatment of trichinosis. *California Med.*, **81:** 348–351.

SOLOMON, C., AND SELIGMAN, B., 1952. Response of trichinosis to ACTH therapy. *New York J. Med.*, **52:** 1444–1446.

STONER, R. D., AND GODWIN, J. T., 1954. The effects of adrenocorticotrophic hormones and cortisone upon acquired immunity to trichinosis in mice. *Am. J. Path.*, **30:** 913–918.

DEVELOPMENT OF RESISTANCE TO MOLLUSCICIDES IN *ONCOMELANIA NOSOPHORA*

BRYCE C. WALTON, M. M. WINN AND J. E. WILLIAMS

406th Medical General Laboratory, APO 343, San Francisco, California

Application of molluscicides has been used rather extensively as a method of controlling schistosomiasis japonica in Japan. Failure in recent years to achieve complete eradication of the intermediate host, *Oncomelania nosophora*, in spite of repeated applications each year of the most effective compounds, has been ascribed in part to the development of resistance to the molluscicides (Okabe *et al.*, 1956; Okabe, 1957). Areas which have received repeated applications for several consecutive years and similar neighboring areas which are known not to have been treated are available within the Kofu endemic area in the Yamanashi Prefecture. Snails from these areas were used in the present study to determine whether resistance had been acquired.

MATERIALS AND METHODS

Snails were collected from localities in the Kofu Valley which had been treated with sodium pentachlorophenate two or three times per year for 7 years (Futaba) and for 4 years (Tamahata), and from one locality that has been treated with dinitro-o-cyclohexylphenol for 6 years (Tanōka). To obviate the possibility that natural differences in resistance might be inherent in different snail populations, control-group snails were collected from three separate areas (Imasua, Kagaminakajo and Tatomi) which had never been treated with molluscicides.

All snails used were collected on the same day and stored dry in Petri dishes at room temperature for several days until tested by exposure to the chemicals in an immersion test developed in this laboratory (Moon *et al.*, 1958). Only snails which were actively moving when placed in aerated tap water were selected for the test. Snails from treated areas and from control areas were tested at the same time under identical conditions.

Test chemicals used were Dowicide "G" (87% sodium pentachlorophenate; Na-PCP) and DN-1, (40% dinitro-o-cyclohexylphenol) furnished through the courtesy of Dow Chemical Company. Concentrations of 1.5 ppm of the active ingredient were used. Each test lot consisted of ten snails. These were placed in 20-ml vials which were then filled with the test solution and covered with a glass slide. Ten snails from each control area were similarly immersed in aerated tap water. All were kept immersed for 6 hours at room temperature. At the conclusion of the exposure, all snails were washed several times with aerated tap water and placed in dry Petri dishes where they were stored at room temperature for 72 hours. At the end of this period the snail shells were crushed lightly, and viability determined on the basis of movement.

RESULTS

The data obtained in the experiment with the snails from two areas that had been treated with sodium pentachlorophenate and from three that were untreated, exposed in the laboratory to 1.5 ppm of this compound for 6 hours, indicated that resistance had not developed (Table 1). The average mortalities in the treated and untreated areas were 31.5 and 31.0 per cent, respectively. In a similar experiment with dinitro-o-cyclohexylphenol, there was a marked difference between the mortality rates of the snails from the treated area and those of the controls (Table 2).

DISCUSSION

Okabe, *et al.*, (1956) compared the LD_{50} of snails, collected from an area in southern Japan (Kyushu) and which had been treated for several years with sodium pentachlorophenate, with that of snails from central Japan where this compound had never been applied. The tests on the two groups of snails were made at different times of the year. Data in this same paper and in one by Tsuda (1952) indicate that susceptibility to molluscicides varies with the time of year. The differences in resistance between snails from the same population, collected during different seasons, were greater than those reported to occur between snails from the treated and snails from

TABLE 1

Mortality of O. nosophora in Immersion Test with Sodium Pentachlorophenate

Immersion Solution	Treated Areas		Untreated Areas		
	(7 years) Futaba	(4 years) Tamahata	Imasua	Kaganakajo	Tatomi
Tap water Control	0/10	1/10	0/10	0/10	0/10
1.5 ppm Na-PCP					
1	3/10	2/10	2/10	1/10	3/10
2	3/10	5/10	5/10	2/10	5/10
3	4/10	3/10	5/10	4/10	5/10
4	4/10	4/10	2/10	5/10	2/10
5	3/10	3/10	3/10	3/10	4/10
6	5/10	4/10	4/10	1/10	5/10
7	2/10	3/10	2/10	4/10	3/10
8	1/10	2/10	2/10	4/10	2/10
9	3/10	4/10	6/10	1/10	2/10
10	2/10	2/10	2/10	1/10	3/10
Total	30	33	33	26	34
% Mortality	30	33	33	26	34

TABLE 2

Mortality of O. nosophora in Immersion Test with Dinitro-o-cyclohexylphenol

Immersion Solution	Treated Area	Untreated Areas	
	Tanõka	Kagaminakajo	Imasua
Tap water Control	0/10	0/10	0/10
1.5 ppm Dinitro-o-cyclohexylphenol			
1	1/10	3/10	4/10
2	0/10	4/10	8/10
3	0/10	2/10	4/10
4	0/10	6/10	4/10
5	0/10	3/10	5/10
6	0/10	1/10	2/10
7	0/10	4/10	6/10
8	1/10	5/10	7/10
9	1/10	5/10	6/10
Total	3	31	46
% Mortality	3.3	34.5	51.1

the untreated areas. In the case of the first paper the geographic location of the areas involved may have introduced additional variables, e.g., differences in strains and in the interval of time between collection and test.

In the present experiments an attempt was made to eliminate as many variables as possible. Under these conditions, in spite of the fact that the areas had been treated with sodium pentachlorophenate for equal or longer periods than the area in Kyushu, there was no evidence of development of resistance. The results obtained with dinitro-o-cyclohexylphenol were markedly different and indicate that the development of resistance to this compound may prove to be a serious handicap to its use in the control of *Oncomelania*.

SUMMARY

Immersion tests were conducted with *O. nosophora* collected from areas treated for several years with sodium pentachlorophenate and dinitro-o-cyclohexylphenol, and from neighboring control areas, to determine if resistance has developed to these molluscicides in the treated snail populations. No resistance to Na-PCP was demonstrated in snails from areas which had been treated for 7 years, and 4 years, respectively. However, there was a marked degree of resistance to dinitro-o-cyclohexylphenol in snails from a locality which had been treated with DN-1 for 6 years.

REFERENCES

Moon, A. P., Frick, L. P., and Asakura, S., 1958. A procedure for laboratory screening of compounds for molluscicidal activity against *O. nosophora*. *Am. J. Trop. Med. & Hyg.*, **7**: 295–297.

Okabe, Koyo, Okahara, T., and Ono, N., 1956. PCP-Na resistance of *Oncomelania nosophora* (Robson). *J. Kurume Med. Assoc.*, **19**(10): 1609–1614 (Japanese text).

Okabe, Koyo, 1957. Schistosomiasis in Japan. *J. Parasitol.*, **43**(Suppl.): 30.

Tsuda, Eizo, 1952. The relationship of the chemical resistance of *O. nosophora* to the seasonal period. *Tokyo Med. J.*, **69**: 48–49 (Japanese text).

BIOCHEMICAL INVESTIGATIONS ON DDT-RESISTANCE IN THE HUMAN BODY LOUSE, *PEDICULUS HUMANUS HUMANUS*

ALBERT S. PERRY AND ANNETTE J. BUCKNER

Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia

The failure of DDT to control the human body louse was first observed in Korea in 1950–51. Laboratory studies conducted by Hurlbut *et al.*, (1952) established that these lice had a measurable degree of physiological resistance. Eddy (1952) confirmed these findings and pointed out that although all samples of lice tested were then highly resistant to DDT, the susceptibility of Korean lice to DDT prior to the use of this insecticide was not known; that is, there was no base line for comparison in that area. Similarly, Barnett and Knoblock (1952) reported that human body lice collected from vagrants in Tokyo were distinctly resistant to DDT and they presumed that these lice had not previously been exposed to DDT, at least for several months prior to testing.

It is impossible to ascertain from these reports whether this resistance was natural or was acquired by selection through the use of DDT. On the other hand, it is an established fact that DDT had been used extensively during a typhus outbreak in Japan and Korea in 1945–46 (Scoville, 1948) and that DDT was subsequently available to the occupation forces.

Starting with a field-collected strain of lice, Yasutomi (1952) was able to select in three generations a resistant strain capable of withstanding residual deposits of 60 to 70 times as much DDT as the original strain. Similar investigations were reported by Kitaoka (1952). Other reports on insecticide resistance in body lice have been published by Busvine (1953) and Hurlbut *et al.*, (1954) and a comprehensive review on this subject has recently been published by Wright and Brown (1957).

The present report covers, in part, the results of a cooperative project with the Orlando, Florida, laboratory of the Entomology Research Division, Agricultural Research Service, United States Department of Agriculture, designed to determine some of the factors involved in the resistance to DDT of the original Korean strain of body lice which has been maintained under DDT selection at that laboratory for several years.

METHODS

Three-to-five-day-old adult lice were obtained regularly, at biweekly intervals, from the Orlando, Florida, laboratory. In a typical test, 25 to 50 lice of either sex were lightly anesthetized with CO_2 and then treated topically with acetone solutions of DDT (dichlorodiphenyl trichloroethane) by means of a microloop delivering a volume of 0.2 μl. Twenty-four hours after treatment, the lice were rinsed with three successive 15-ml portions of acetone to remove the unabsorbed DDT. The lice were then ground to a fine powder with anhydrous Na_2SO_4 and extracted with ether in a Soxhlet apparatus for 8 hours. Following evaporation of the ether, the residue was chromatographed through a H_2SO_4-Celite column (Davidow, 1950) using CCl_4 as eluant. DDT and breakdown products were determined by the Schechter-Haller (Schechter *et al.*, 1945) method.

The amount of DDT applied was determined for each test by extracting and analyzing the insecticide immediately after its application to 25 to 50 lice.

In several tests where radioactive DDT (DDT-C^{14}) was used, the treated lice were first rinsed as described above and then placed directly in the oxidizing mixture used in the estimation of C^{14} (Communicable Disease Center, 1954). This direct oxidation obviates the necessity of macerating and extracting the lice. In similar exploratory tests, when using the Schechter-Haller colorimetric procedure, the DDT-treated lice were placed directly in the nitrating mixture without prior extraction. In this case, it was found necessary to increase the amount of nitrating mixture to 20 ml; and to prolong the nitration period to 2 hours.

Because of some difficulties encountered with the topical application method, a search was made for a commercially-available membrane which might be suitable for the artificial feeding of lice on blood to which DDT and various other insecticides could be added. None of the mem-

TABLE 1

Absorption and metabolism of DDT by 3- to 5-day-old adult DDT-susceptible and DDT-resistant body lice at 24 hours following the topical applications of various dosages of DDT in acetone. Lice in untreated controls showed no mortality.

Strain and colony designation	Lice	Sex	DDT applied μg/louse	Recovered (μg/louse)			24-hour mortality %
				external DDT	internal		
					DDT	DDE	
Susceptible	100	M & F	0.20	0.10	0.0	0.0	98
	75	M	0.82	0.58	0.04	0.0	100
	73	F	0.64	0.61	0.06	0.0	97
	50	M	1.00	1.07	0.02	0.0	100
	50	F	1.23	1.06	0.03	0.0	98
	95	F	1.20	1.26	0.0*	†	100
	25	M	2.08‡	1.53	0.07	†	100
	25	F	2.08‡	1.44	0.19	†	100
Resistant:							
Colony "A"§	50	M & F	1.18	1.23	0.0	0.0	2
	40	M	2.95	2.70	0.03	0.0	42
	44	F	3.55	3.10	0.07	0.0	29
Colony "B"‖	98	F	2.16	1.54	0.0*	†	20
Colony "C"**	50	M	1.58	1.09	0.0	0.0	8
	49	F	1.32	1.23	0.14	0.0	0
	25	F	1.90	2.10	0.0*	0.0†	0
	25	M	2.16‡	1.53	0.04	†	0
	25	F	1.65‡	1.30	0.08	†	0

* Extracts bioassayed against *Aedes aegypti* larvae.

† Test does not distinguish between DDT and DDE.

‡ Radioactive DDT.

§ Reared continuously on cloth treated with 0.01 per cent DDT.

‖ Reared for 17 generations on cloth treated with 0.01 per cent DDT and subsequently reared on untreated cloth for 25 generations.

** Taken in the egg stage from Colony "A" and transferred to untreated cloth for rearing to adult stage.

branes tested[1] was found satisfactory for feeding lice; consequently, the method of Fuller, *et al.*, (1949) was adopted.[2]

Briefly, the technique employed was as follows: A chick 2 to 5 days old was killed with chloroform, and depilatory cream was applied to the sides and breast, covering as much surface as possible. Ten minutes after application, the cream was washed off with lukewarm water. The skin was then swabbed with 70 per cent alcohol, followed by acetone, after which it was carefully dissected from the chick. The section of skin removed was drawn tightly over one end of a glass cylinder (1 inch diameter, 2.5 inches long) with the downy surface facing the inside of the cylinder. The skin was sealed to the glass with paraffin. The cylinder was lowered into a 20-ml beaker containing 5 to 10 ml of citrated human blood and held rigidly in place with a spring clamp in such a way that the membrane did not touch the bottom of the beaker. The beaker was then lowered into a temperature-controlled water bath. Fifty adult lice on a small piece of felt were lowered gently to the bottom of the cylinder where they were allowed to feed for 1 hour.

DDT-C^{14} was incorporated in the blood as follows: 1 ml of ethanol containing DDT-C^{14} was diluted with 9 ml of a 5 per cent aqueous solution of acacia, thus forming a suspension which was

[1] The following membranes were tried: Silverlite, Baudruche Transparent, Single Zephyr, Double Zephyr, Opaque White, Yellow Transparent, and Black; products of Long and Long Company, Belleville, New Jersey.

[2] A useful technique using gutta percha membranes for the artificial feeding of lice, published by Haddon (1956a, 1956b) has come to the authors' attention too late for inclusion in the present experiments.

stable for several hours. One ml of this suspension was diluted with 9 ml of citrated human blood and the mixture was homogenized in a blender for 1 minute. The blood was then transferred to the beaker and maintained at 36° C ± 1°. The maintaining of the blood at this constant temperature was a critical factor in the successful feeding of lice.

DDT intake, a function of the amount of blood consumed, was determined by weighing the lice before and after feeding. In some cases, a batch of lice was combusted immediately following feeding and the amount of DDT determined radiometrically.

RESULTS

Topical application. The results of tests with the susceptible and DDT-resistant strains of lice are shown in Table 1. It is evident that the resistant strains can tolerate larger amounts of DDT with no apparent harmful effects. A conspicuous feature of these results is the apparent slow absorption of DDT by both resistant and susceptible strains. (Microscopic examination revealed that the louse cuticle is wetted by acetone with difficulty, hence it might be assumed that no appreciable amount of DDT was removed from the internal tissues by the acetone wash). In most cases, the amounts of DDT absorbed in 24 hours were so small that quantitative determinations fell below the limit of sensitivity of the colorimetric method. Radiometric assays of extracts of DDT-C^{14}-treated lice showed small amounts of radioactivity present.

Since most of the DDT applied was recovered in the external rinse (Table 1) it appears that the amount of DDT necessary to kill susceptible lice is indeed small. From this standpoint, it would be difficult to make quantitative comparisons between the resistant and susceptible strains in terms of physiologically-active DDT present within the tissues. It also is evident (Table 1) that no trace of DDE (dichlorodiphenyl dichloroethylene) was detected in extracts of either strain, but this finding, too, has its limitations inasmuch as the quantities involved were too small for colorimetric analysis.

Observations made during these tests showed that characteristic symptoms of DDT-poisoning appeared in susceptible lice shortly after application of the insecticide, but these were totally absent (or latent at higher dosages) in the resistant strains. Since no marked differences between resistant and susceptible lice were found in regard to penetration of the insectide, rate of absorption can be ruled out as a defense mechanism against the action of DDT in the resistant strain.

Ingestion. The averaged results of two to six tests are shown in Table 2. It is clear that the resistant strain can tolerate much larger quantities of ingested DDT than the susceptible strain. At a concentration of 10 ppm of DDT in the blood, the susceptible lice exhibited symptoms of poisoning between 30 and 60 minutes after feeding. With 25 ppm, the onset of symptoms was evident immediately after removal from the feeding chamber, and manifestations of excitement, tremors, loss of coordination, strong peristaltic movements and loss of locomotory control were prominent. The 24-hour mortality of susceptible lice was 90 per cent. In contrast, the resistant lice showed no signs of hyperactivity, nor any other characteristic symptom of DDT-poisoning at a concentration of 100 ppm. Only 10 per cent mortality resulted in 24 hours which was equivalent to that of the controls.

The quantities of DDT ingested depend on the amount of blood consumed. On the average, the susceptible strain consumed 0.5 mg of blood per louse and the resistant strain 0.7 mg per louse during the 1-hour feeding period. Considering an average body weight of 1.2 mg per louse (on a basis of 50:50 male to female ratio), when engorged, the resistant louse consumes a meal approximately 50 to 60 per cent of its body weight.

Based on the amount of DDT ingested, the LD_{50} and LD_{90} for the susceptible strain were found to be approximately 0.005 and 0.011 μg DDT per louse, respectively. A dosage of 0.081 μg DDT per louse produced only 10 per cent mortality in the resistant strain. The latter failed to feed on blood containing 250 and 500 ppm of DDT. The reason for this failure has not been ascertained.

In another series of feeding tests with resistant lice involving combinations of 50 ppm DDT with 50 ppm of either 1,1-bis-(*p*-chlorophenyl) methyl carbinol (DMC), bis-(*p*-chlorophenyl) chloromethane (MR-60), or *p*-bromobenzenesulfonotoluidide (Table 2), only a slight increase in mortality was observed which may have been due to an additive effect. While these compounds are among the most powerful DDT-synergists

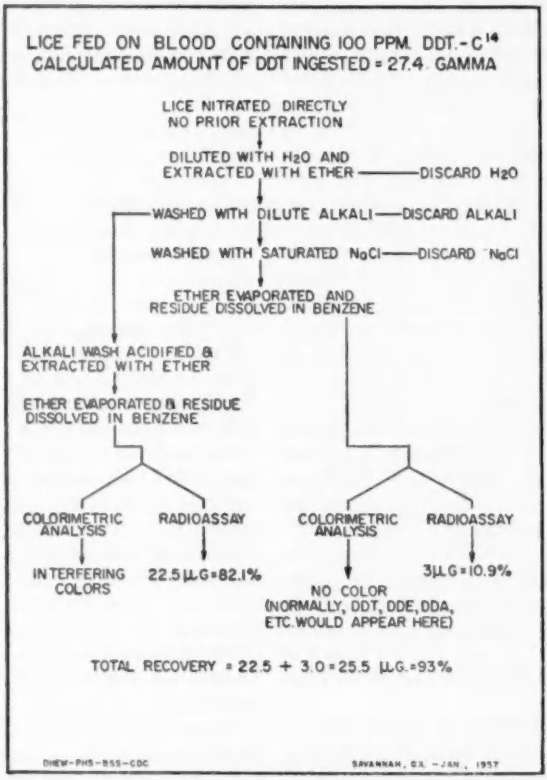


FIG. 1. Schematic Diagram of a Typical *in vivo* Analysis for DDT and Metabolites

against DDT-resistant house flies, they apparently were relatively ineffective against DDT-resistant lice.

DDT-metabolism. The chick-skin feeding technique was employed also in studying the metabolism of DDT in resistant and susceptible lice. In a typical test with the resistant strain, 600 to 800 lice were fed on citrated human blood containing 100 ppm DDT-C^{14}. Twenty-four hours after feeding, the lice were macerated in a mortar with sea sand and anhydrous sodium sulfate and extracted with ether in a Soxhlet apparatus for 8 hours.

Radiometric assays of extracts such as those described above showed only 10 to 20 per cent recovery of the calculated amount of radioactivity present. Extraction with other solvents such as benzene, chloroform, carbon tetrachloride or n-hexane did not improve the recovery. Extraction with methanol yielded 40 to 50 per cent recovery of the total radioactivity. However, when the lice were digested directly in the oxidizing mixture used in radioassay, 80 to 90 per cent of the radioactivity was recovered. This indicated that the ingested DDT was metabolized into a polar substance which was not soluble in common organic solvents.

Owing to the small amounts of insecticide involved in relation to total extractives, extraction of the metabolite with more polar solvents did not prove suitable for colorimetric analysis by the Schechter-Haller method. Hence, groups of approximately 200 lice were placed directly in 20 ml of nitrating mixture (1 part concentrated H_2SO_4 to 1 part fuming HNO_3 by volume) and nitrated for 2 hours. The final step in the colori-

metric procedure showed no color indicative of DDT or any metabolite thereof. Radioassay of the nitrated product yielded only 4 to 10 per cent of the calculated radioactivity. It was subsequently found that the major portion of the nitrated metabolite was removed from the ether phase by the alkali wash which is normally discarded, and that it could be recovered if the alkali wash was first acidified and then re-extracted with ether. Under these conditions it was possible to account for most of the radioactivity, but the presence of large amounts of interfering materials which could not be eliminated with common adsorbents without loss of the metabolite prevented a clear-cut analysis by colorimetric means. A schematic diagram of a typical analysis is shown in Figure 1.

The fact that the nitrated material is removed from ether solution by dilute alkali indicates that it is neither DDT, DDE, nor DDA (dichlorodiphenyl acetic acid) since the nitrated products of the latter compounds are not soluble in alkali. It is worth noting that of several compounds tested which respond to the Schechter-Haller method, only *p*-chlorobenzoic acid was found to be removed by dilute alkali following nitration. This is not to infer, however, that the louse metabolite is similar to this compound on the basis of this property alone.

It is quite conceivable that the metabolite acquires its polarity by virtue of its conjugation with a protein or a lipoprotein since approximately 50 per cent of the total radioactivity which was not solvent-extractable appeared to be associated with particulate matter. Subjecting these particles to acid or alkali hydrolysis failed to release the metabolite in soluble form.

Inasmuch as the quantity of metabolite available for study was very small, attention was directed to isolation of the detoxifying mechanism in vitro. Tissue homogenates were prepared in saline:bicarbonate and aliquots were incubated in a Warburg apparatus. DDT-C^{14} was added in small volumes of ethanol, glutathione was placed in the side arm and the flask flushed with N_2. After incubation at 37° C for 4 hours, 70 to 80 per cent of the DDT was converted to a water-soluble metabolite with the same solubility characteristics as that obtained in vivo. However, the metabolite obtained by enzymatic conversion in vitro was not completely removed by dilute alkali in the Schechter-Haller procedure as was the case in vivo, and, in addition, it formed a red-colored complex with sodium methylate which is indicative of a DDT-metabolite. Homogenates of both susceptible and DDT-resistant lice showed approximately the same degree of DDT-metabolism in vitro.

Details of the enzymatic conversion of DDT by susceptible and DDT-resistant lice, and also a study of the kinetics of the reaction will be the subject of another paper.

DISCUSSION

A conspicuous feature of the results obtained with topical application is the extremely slow rate of absorption of DDT through the louse cuticle. Owing to the particularly tough and oily nature of this cuticle solvents such as ethanol, acetone, or benzene proved unsuitable for topical application. Some higher alcohols were too toxic, and several vegetable and mineral oils, including Risella oil, were found unsatisfactory.

Since no marked differences in absorption were found between resistant and susceptible lice, rate of penetration of DDT may be ruled out as a defense mechanism of the resistant strain. It also is evident that only minute amounts of DDT were needed internally to cause complete mortality of the susceptible strain.

Greater differences in tolerance for DDT are clearly demonstrated in the feeding experiments given in Table 2. The fact that resistant lice could tolerate 100 ppm of DDT in the blood with minimum mortalities, whereas 10 ppm were lethal to more than 50 per cent of the susceptible lice, indicates the presence of an intrinsic biochemical difference and strengthens the conclusion that penetration of DDT through the cuticle is not an important factor.

The metabolism tests in vivo indicated that the ingested DDT was almost completely metabolized by the resistant strain whereas little or no degradation of DDT occurred in the susceptible strain. While a concentration of 10 ppm of DDT produced 53 per cent mortality of the susceptible lice in 24 hours, the limit of sensitivity of the analytical methods did not permit the use of lower concentrations in order to ascertain whether or not susceptible lice could metabolize sublethal doses of DDT.

Although the metabolite obtained from the in vivo metabolism of DDT by the resistant

strain has not been positively identified, certain of its properties have been clearly defined. On the basis of solubility characteristics, both before and after nitration, DDE might be ruled out as a possible metabolite, although it is conceivable that conjugation with a protein or a lipoprotein might sufficiently alter the solubility of DDE to form a more polar conjugate.

In limited experiments, using topical applications of DDT to the same strain of resistant lice mentioned in this paper (colony A, Table 1), Kearns (1955) reported the presence of a metabolite that responded in the same manner as DDE to the Schechter-Haller test. On the other hand, tests in vitro, using lice homogenates, showed no evidence of the existence of a mechanism comparable to that found in the resistant house fly. Kearns stated, however, that owing to difficulties encountered in extraction and in removal of interfering materials, these findings were not conclusive.

It is worth noting that none of the synergists tested in combination with DDT appreciably increased the mortality of the resistant strain. These findings are in agreement with the results of Cole, *et al.*, (1957) who reported no increase in the mortality of DDT-resistant lice exposed to DDT:DMC and DDT:MR-60 combinations. Other investigations have demonstrated that certain DDT-synergists, including those mentioned above, interfere with the enzymatic conversion of DDT to DDE in house flies, both in vivo (Perry and Hoskins, 1950; Perry *et al.*, 1953) and in vitro (Moorefield and Kearns, 1955). These results strengthen the assumption that the metabolite from lice is not DDE and that the mechanism of DDT-breakdown is different from that found in flies.

The experiments in vitro, which will be reported in detail in another paper, distinctly showed that homogenates and acetone powders of both resistant and susceptible lice are capable of metabolizing DDT at an approximately equal rate. By analogy with the enzyme DDT-dehydrochlorinase which is found in DDT-resistant house flies but not in any measurable quantity in susceptible strains (Sternburg, *et al.*, 1954), one might infer that enzymatic breakdown of DDT in lice is a natural phenomenon, i.e., that all strains of lice, both resistant and susceptible, possess a "hidden resistance potential" to DDT in the form of a detoxifying enzyme system whose action on DDT is interfered with by some other, unknown, biochemical factor which is operative in the susceptible strain but is by-passed in the resistant strain. This by-pass mechanism which is accentuated through selection pressure with DDT, would then allow the resistant louse sufficient survival time to enable the enzyme to neutralize the absorbed insecticide. In order to arrive at a logical conclusion, these hypotheses must be tested further by investigating several other susceptible and DDT-resistant strains from various localities.

TABLE 2

Effect of ingested DDT and DDT-synergist combinations in blood on susceptible and resistant lice. Results are averages of 2 to 6 tests with each concentration. Each test represents 25 to 50 lice.

Strain	DDT in blood (*ppm*)	Blood ingested (mg/louse)	Calculated amount of DDT ingested (μg/louse)	24-hour mortality (%)
Susceptible	10	0.56	0.005	53
	25	0.42	0.011	90
	50	0.41	0.020	100
	100	0.53	0.053	100
	100	0.40	0.040*	100
	0	0.58	—	15
Resistant Colony "A"	25	0.63	0.016	5
	50	0.50	0.025	8
	100	0.81	0.081	10
	50 DDT	0.45	0.022 DDT	17
	50 DMC†		0.022 DMC	
	50 DDT	0.83	0.041 DDT	30
	50 MR-60†		0.041 MR-60	
	50 DDT	0.97	0.048 DDT	30
	50 p-BST‡		0.048 p-BST	
	0	0.57	—	10

* Radiometric assay of the treated lice showed the presence of 0.041 μg/DDT per louse.

† See text.

‡ *p*-bromobenzenesulfonotoluidide.

SUMMARY

DDT was found to be absorbed very slowly from acetone or benzene solutions when applied topically to susceptible and DDT-resistant human body lice. The resistant strain could tolerate larger quantities of applied DDT without any appreciably harmful effect. The feeding of lice through dissected chick skins on citrated human blood containing various concentrations of radioactive DDT showed that the resistant

strain could tolerate 100 ppm of DDT in the blood whereas 10 ppm caused 53 per cent mortality in the susceptible strain.

Only resistant lice metabolized DDT in vivo to a relatively nontoxic material unextractable with common organic solvents but partly extractable with methanol. Determination of the metabolite by the Schechter-Haller method yielded a nitrated product which was removed from solution by dilute alkali, unlike DDT, DDE, or DDA.

Both susceptible and DDT-resistant lice metabolized DDT in vitro at approximately the same rate. The nontoxic, water-soluble metabolite gave a red-colored complex in the Schechter-Haller procedure and had solubility characteristics similar to that obtained in vivo.

It appears that an intrinsic biochemical factor which, presumably, interferes with the detoxifying mechanism, might be operative in the susceptible strain. This interference is manifested in vivo but not in vitro.

REFERENCES

Barnett, C. H., and Knoblock, E. C., 1952. Chemical and biologic studies on DDT resistance of lice. *U. S. Armed Forces Med. J.*, **3:** 297–304.

Busvine, J. R., 1953. Forms of insecticide resistance in houseflies and body lice. *Nature*, **171:** 118–9.

Cole, M. M., Couch, M. D., Burden, G. S., and Gilbert, I. H., 1957. Further studies on resistance of human body lice to insecticides. *J. Econ. Entomol.*, **50:** 556–9.

Communicable Disease Center, Technical Development Laboratories, Public Health Service, U. S. Dept. Health, Education, and Welfare, Savannah, Ga., 1954. *Chem. Memo.* 3.

Davidow, B., 1950. Isolation of DDT from fat. *J. Assoc. Official Agr. Chem.*, **33:** 130.

Eddy, G. W., 1952. Effectiveness of certain insecticides against DDT-resistant body lice in Korea. *J. Econ. Entomol.*, **45:** 1043–51.

Fuller, H. S., Murray, E. S., and Snyder, J. C., 1949. Studies of human body lice *Pediculus humanus corporis*. *Pub. Hlth. Rpts.*, **64:** 1287–92.

Haddon, W., Jr. 1956a. An artificial membrane and apparatus for feeding of the human body louse, *Pediculus humanus corporis*. *Am. J. Trop. Med. & Hyg.*, **5:** 315–25.

Haddon, W., Jr., 1956b. The maintenance of the human body louse, *Pediculus humanus corporis* through complete cycles of growth by serial feeding through artificial membranes. *Am. J. Trop. Med. & Hyg.*, **5:** 326–30.

Hurlbut, H. S., Altman, R. M., and Nibley C., Jr., 1952. DDT resistance in Korean body lice. *Science*, **115:** 11–12.

Hurlbut, H. S., Peffly, R. L., and Salah, A. A., 1954. DDT resistance in Egyptian body lice. *Am. J. Trop. Med. & Hyg.*, **3:** 922–9.

Kearns, C. W., 1955. The enzymatic detoxication of DDT. In "*Origins of resistance to toxic agents*" edited by Sevag, M. G., Reid, R. D., and Reynolds, O. E., Academic Press, Inc., New York City. pp. 148–59.

Kitaoka, M., 1952. DDT-resistant lice in Tokyo. *Japan J. Med. Sci. and Biol.*, **5:** 75–88.

Moorefield, H. H., and Kearns, C. W., 1955. Mechanism of action of certain synergists for DDT against resistant house flies. *J. Econ. Entomol.*, **48:** 403–6.

Perry, A. S., and Hoskins, W. M., 1950. The detoxification of DDT by resistant house flies and inhibition of this process by piperonyl cyclonene. *Science*, **111:** 600–1.

Perry, A. S., Mattson, A. M., and Buckner, Annette J., 1953. The mechanism of synergistic action of DMC with DDT against resistant house flies. *Biol. Bull.*, **104:** 426–38.

Schechter, M. S., Soloway, S. B., Hayes, R. A., and Haller, H. L., 1945. Colorimetric determination of DDT. Color test for isolated compounds. *J. Ind. & Eng. Chem., Anal. Ed.*, **17:** 704–9.

Scoville, A. B., Jr., 1948. Epidemic typhus fever in Japan and Korea. In "*Rickettsial Diseases of Man*", a symposium published by the AAAS, Washington, D. C., Edited by E. R. Moulton. pp. 28–35.

Sternburg, J., Kearns, C. W., and Moorefield, H., 1943. DDT-dehydrochlorinase, an enzyme found in DDT-resistant flies. *J. Agr. & Food Chem.*, **2:** 1125–30.

Wright, J. W., and Brown, A. W. A., 1957. Survey of possible insecticide resistance in body lice. *Bull. World Health Organ.*, **16:** 9–31.

Yasutomi, Kazuo, 1952. Studies on insect-resistance to insecticides. I. Development of resistance of human body louse, *Pediculus humanus corporis* de Geer, to DDT and BHC. *Botyu-Kagaku*, **17:** 41–4.

AN INFESTATION OF A HUMAN HABITATION BY *DERMANYSSUS GALLINAE* (DEGEER, 1778) (ACARINA: DERMANYSSIDAE) IN NEW YORK CITY RESULTING IN SANGUISUGENT ATTACKS UPON THE OCCUPANTS

ROGER W. WILLIAMS

School of Public Health and Administrative Medicine, Columbia University

Dermanyssus gallinae (DeGeer, 1778) is an important blood-sucking pest of certain birds, especially chickens, turkeys, canaries, pigeons, swallows, sparrows and towhees. Like the bedbug it is nocturnal in habit, feeding at night and retiring into the nest or cracks and crevices of coops and cages during the day. Heavy infestations of these mites not infrequently kill the avian host.

There is no doubt that this mite may cause dermatitis in man but whether these conditions are due to the bite or a sensitivity to the mites, or to certain products of the mites, is not clear. There is disagreement in pertinent literature regarding the feeding of *D. gallinae* on the blood of man. Of some 12 books in general use which were consulted, three used the word "bite;" five the word "attack," which implies biting; two were noncommittal, using such expressions as "irritate" or "attendants of fowl—receive their attention." One stated, "While man is commonly annoyed by crawling mites, instances of actually having been bitten (by *D. gallinae*) appear to be quite rare," and another commented, "Though the mites cause a marked dermatitis in humans, it is said that they do not obtain human blood." Wisseman and Sulkin (1947) state, "From time to time reports of chicken and bird mites (*D. gallinae*) attacking man have appeared in the medical literature. In only one instance (Boyt, 1937) has the attacked individual or his associates actually noted engorgement of the mites. This information was obtained second-hand by the author. Our findings concur with those of Wood (1917) in that though mites crawling on the skin produce a disagreeable sensation, none have ever been observed to engorge or bite. This does not exclude lesions produced by products of the mites on sensitive individuals." Roman, LeCoarer and Vital-Durand (1950) describe two cases of human infestations by *D. gallinae* in France but in neither of these cases could they ascertain whether the clinical manifestations were the results of bites or, indeed, whether the mites were biting the individuals at all. These authors discuss the controversy as it appears in the French literature. They cite Guiart (1910) and Louste and Levy-Franckel (1936) as stating that *D. gallinae* will bite man and Brumpt (1949) as claiming that this species of mite will never ingest human blood.

Toomey (1921) discusses the clinical pathology of gamasoidosis (fowl-mite dermatitis caused by *D. gallinae*) and presents a bibliography of about 50 references, most of which are 19th century works. In reference to feeding on man, Toomey indicates that the mites do not burrow into the epidermis but remain in sweat and sebaceous ducts. Their presence here causes the ducts to become inflamed and gape slightly. "... it is virtually certain that *Dermanyssus* feeds, when on the skin of man, by sucking through the soft walls of the ducts." He does not suggest whether the food is blood or secretions from the glands.

Sulzberger and Kaminstein (1936) reviewed the literature on dermatitis caused by bird mites and indicated that as far as they could ascertain there were no recent reports of human eruptions due to avian mites appearing in the American and English literature. They then reported on cases that occurred in two families in New York City. They concluded with the following remarks: "The common bird-mite (*D. gallinae*) is a blood-sucking gamaside which occurs wide-spread on wild and domestic fowls. In spite of the fact that this mite is an obligatory parasite of birds and cannot continue to live and propagate on man, it nevertheless occasionally causes itching eruptions in those susceptible human beings who, in various ways, come into more or less intimate contact with the mite from the bird." Mention of ingestion of human blood was carefully avoided. These authors attempted to ascertain whether persons affected by the mites have a skin hypersensitivity to the products of these mites. It

was concluded that there was no evidence of hypersensitivity demonstrable by means of their experiment.

DeOreo (1958) describes cases of mite dermatitis which were said to be caused by the "bite" of *D. gallinae*. The criteria used to incriminate this mite as a biting pest of man were the presence of a characteristic cutaneous skin reaction, typical of an insect bite; close proximity of pigeons' nests; disappearance of the cutaneous manifestations after removal of nests and use of acaricides; and identification of the mite as *D. gallinae*. No mites were seen to engorge on human blood and no direct evidence was presented that any mites had ingested human blood.

Thus, it becomes apparent that there is a wide divergence of opinion regarding the feeding of *D. gallinae* on man. Some believe that it does not, and will not, ingest human blood but are unable to account for the dermatitis that this mite produces on man other than to hypothesize that the reaction is an allergic one appearing on hypersensitive individuals. Those who state that this mite does ingest human blood do so on the basis of circumstantial rather than factual evidence since the only report of this mite biting man (Boyt, 1937) is based on indirect information and concerned only a single mite. The evidence presented by the present writer indicates that those who have assumed that *D. gallinae* will feed on man are correct.

FACTORS LEADING TO INFESTATION OF A NEW YORK APARTMENT

Pigeons, a common host of *D. gallinae*, abound throughout various areas of New York City and are usually found in greatest numbers where the human population feed them well. Here they roost and nest on the roofs, ledges, and window sills of buildings. The third-floor apartment under consideration is in a building located in an area in which the pigeon population is larger than average for it has been the daily habit of two women, for the past 20 years, to feed the pigeons stale bread and cake from a nearby lunch room at approximately the same hour.

An addition to an adjoining building, constructed some 18 months previously, brought the flat roof of the new extension against the outside wall of the apartment 12 inches below the bathroom window sill. On this flat but somewhat uneven roof, water accumulated in small pools following rains and pigeons congregated near the window to drink from the pools. The bathroom window was rather loose fitting for, when closed and locked, a considerable amount of dirt and soot could blow in under it within 12 to 24 hours. Around this loose-fitting window the mites apparently gained access to the apartment.

ATTACK ON THE INHABITANTS

Late in November, 1957, a few mites were observed by the occupants of the apartment, crawling on the bathtub and white-tile bathroom walls. These were crushed and no particular attention was paid to them. About 6 weeks later the occupants noted that they were being bitten, presumably by insects. However, these bites were not associated with the mites until small fresh blood spots were observed on the bed sheets upon arising in the mornings. These spots proved to be crushed mites which had recently fed and which were mashed by the movements of the human hosts. On January 24, 1958 when the bathmat was removed from the bathroom floor about 50 mites were found under it. Later they were found behind towels hanging in the bathroom and under the hall rug near the bathroom door. Since mites were never found in the bedroom except on the bed it was inferred that mites were transported mechanically from the bathroom to the bed on the bodies or clothing of the human hosts.

About mid-February the author was given a number of these mites to identify and they were found to be *D. gallinae*. At that time he examined the blood from the digestive tract of a number of the mites, some of which had been collected from the bed. The erythrocytes were the round, biconcave, anucleate cells of mammalian blood and not the somewhat elongated, nucleate cells of avian blood. This fact would appear to be fairly good evidence, along with the blood spots on the sheets, that these mites will, on occasion at least, ingest human blood. Whether or not they can survive and multiply on this diet is not known. However, the occupants volunteered the information that at first the mites they recovered in the apartment and from themselves were small but that during the month of February numbers of large ones, as well as small ones, were collected.

The bites on the male occupant occurred primarily on the ankles, in the popliteal and antecubital fossae, and in the axillary region. The

irritation from these bites was said to be intense for 3 to 4 days. The female occupant, although bitten on the body, complained more of an itching scalp and at times combed numbers of these mites from her hair into the bathtub where they could be seen crawling about. Some difficulty was encountered in eliminating the mites from the hair. Larkspur lotion (three 2-ounce bottles), and two applications of A-200 Pyrinate had been used without success. Living mites were combed from the hair within a few hours of application. Benzylbenzoate was suggested by the author and this gave considerable relief from the prurience and only one mite was recovered from the hair 3 days after its use. This mite probably gained access to the hair after the benzylbenzoate had been washed out.

ERADICATION OF THE MITES FROM THE APARTMENT

Professional exterminators and housing officials jointly participated in determining the control and eradication procedures. First applications of insecticides and acaricides were made during the last week in January. The last mites were recovered during the third week in February. Chlordane and Malathion were applied to the bathroom floor and walls, to the hall floor, and to rugs in the bedroom and hall. The mattress on the bed was sprayed with DDT solution. All windows facing the roof from which the mites appeared to have gained entrance were caulked from the outside and crevices between tiles in the bathroom floor were filled with grout. It was planned to attempt to level the new adjacent roof in an effort to eliminate standing water which attracted the pigeons. Trapping of pigeons on this roof was also contemplated.

SUMMARY

Although a marked clinical dermatitis is common in some individuals that become closely associated with the bird mite, *Dermanyssus gallinae*, the literature presents little evidence that this mite will ingest human blood and many investigators feel that this species will never ingest it. The invasion of a New York City apartment by these mites resulting in attacks on the occupants is described. The finding of mammalian erythrocytes in the digestive tract of mites collected from this apartment, some of which were taken from the bed of the occupants, as well as the appearance of fresh blood splotches on the bed sheets resulting from crushed mites which had recently fed, offer some factual evidence that *D. gallinae* may, on occasion at least, partake of human blood.

ACKNOWLEDGMENTS

The author wishes to express his thanks to the Columbia University faculty member and his wife who occupied the apartment invaded by *D. gallinae* and to Mr. Ted Farris, Assistant to the Director of Residence Halls, Columbia University, for most of the epidemiological data presented.

REFERENCES

Boyt, R. H., 1937. *Dermanyssus* and *Liponyssus avium* et *gallinae* attacking man. *Brit. J. Dermatol. and Syph.*, **49:** 66–68.

Brumpt, E., 1949. *Précis de Parasitologie*, Paris, 6ed.

DeOreo, G. A., 1958. Pigeons acting as vector in acariasis caused by *Dermanyssus gallinae* (DeGeer, 1778). *A.M.A. Arch. Dermatol.*, **77:** 422–429.

Guiart, J., 1910. *Précis de Parasitologie*, Paris.

Louste, A., and Levy-Franckel, A., 1936. *Parasites animaux*. N. Prat. Dermat. (Darier *et al.*) **2:**, pp. 1–98.

Roman, E., Le Coarer, R., and Vital-Durand, 1950. Deux cas d'infestation humaine par des acariens du genre *Dermanysse* observes dans l'est Lyonnais. *Bull. Mens. Soc. Linn. de Lyon.*, **19:** 179–180.

Sulzberger, M. B., and Kaminstein, I., 1936. Avian itch mites as a cause of human dermatosis. *Arch. Dermatol. and Syph.*, **33:** 60–72.

Toomey, N., 1921. Gamasoidosis, fowl-mite dermatitis. *Urol. and Cut. Rev.*, **25:** 705–710.

Wisseman, C. L., and Sulkin, S. E., 1947. Observations on the laboratory care, life cycle, and host of the chicken mite, *Dermanyssus gallinae*. *Amer. J. Trop. Med.*, **27:** 463–468.

Wood, H. P., 1917. The chicken mite; its life history and habits. U. S. Dept. Agric. Bull. No. 553.

INTESTINAL PROTOZOA AND HELMINTHS IN THE PEOPLES OF EGYPT LIVING IN DIFFERENT TYPE LOCALITIES[1]

R. E. KUNTZ, D. K. LAWLESS, H. R. LANGBEHN, AND G. M. MALAKATIS[2]

Naval Medical Research Unit Number 2, Taipei, Taiwan

In conjunction with a rather extensive program on the study of the epidemiology and biological factors relating to disease in Egypt, a number of surveys were made to determine the incidence of intestinal protozoa and helminths in the Egyptians. Some of these surveys dealt with problems in specific type villages (Wells and Blagg, 1956), some (Weir, *et al.*, 1952; Chandler, 1954) were concerned with the influence of sanitation upon parasite propagation and disease control, while others (Kuntz and Lawless, 1958) revealed the intestinal fauna and host-parasite relationships in a young-age (infants to young adults) segment of a typical village population.

The present report is based upon a study of 1,408 fecal specimens taken during the course of field studies in widely separated localities in Lower and Upper Egypt. Collections were made as opportunity permitted over a period of approximately 2 years. Different type localities were selected in order to provide stool specimens from peoples living under different conditions in representative areas.

MATERIALS AND METHODS

Stools were obtained with the assistance of village omdas (lord-mayors), school teachers and headmen of Bedouin camps who summoned subjects desired for our study. In most cases individuals were handed an empty sputum cup and asked to step into a private room, i.e., in a school or in a village clinic, to produce a study specimen. Bedouins usually looked for a desert shrub or took refuge on the far side of a camel. This method of approach, i.e., on an individual basis, reduced to a minimum the substitution of animal feces, or the splitting of a single sizable fecal specimen to satisfy our requests. It may be of interest to state that over 90 per cent of our requests were honored within a period of 5 to 15 minutes.

As in our previous studies the Merthiolate-iodine-formalin (MIF) (Sapero and Lawless, 1953) preservation method was employed and materials were fixed soon after passage to permit effective detection of intestinal protozoa. Several samples of feces the size of a garden pea were selected from different areas of each stool, placed in MIF solution, and were well macerated with an applicator stick in a 15-ml-capacity screw-cap vial. A second vial was used for preservation of an equal quantity of feces in 6 per cent Formalin. After return to the laboratory in Cairo the top of vialed specimens was sealed by dipping into a heated mixture of wax and paraffin. Specimens were studied later as time and opportunity allowed.

A 10- to 15-minute search was made of a single direct MIF smear consisting of several drops (22 x 22 mm. cover slip) from a sample of the upper layer of sediment of each vial. Although this type of sample represents considerable concentration by the sedimentation principle, especially if the vial stands undisturbed for an hour before a sample is withdrawn, Formalin-fixed samples were obtained and subjected to the AMS-III technic of Hunter *et al.*, (1948) for concentration of helminth eggs.

Over 75 per cent of our samples were obtained from school children, i.e., 8 to 14 years of age and young adults. For convenience in study the 13 villages may be grouped into the following categories representing different, general type localities.

Coastal fishing village. El Burg (Fouadiya Province), is a small village of the Delta of Lower Egypt located on the northern shores of brackish-water Lake Burullus, 1/8 mile inland from the Mediterranean Sea. Only a few of the several thousand persons are engaged in farming. The population on the whole consists of fishermen or people involved in the fishing industry. These peoples are great fish eaters and supply the mar-

[1] The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of the Navy.

[2] The authors are indebted to Abdel Aziz Salah and Said Kassim Mohamed of NAMRU-3, Cairo, Egypt for assistance in collection of study specimens and to Dean E. Armstrong, HM1, USN for assistance in examination of materials.

kets of Lower Egypt and Cairo with fresh and salted fishes. Flies are numerous and poor sanitary conditions exist throughout the village.

Farming communities. Villages examined were *Abu Rawâsh* and *Tanâsh* (Giza Prov.), *Sindbis* (Qalyubiya Prov.) and *Shirbin* (Gharbiya Prov.), all in the Nile Delta, and *El Faiyum* which is located in the central part of Faiyum Province. The peoples of these villages are primarily concerned with different phases of farming. Irrigation canals run through the villages, flies abound in and about the homes, and sanitary conditions are classed as poor.

Urban communities. These were *Alexandria* (Alexandria Governorate) on the Mediterranean coast, *Cairo* (Cairo Governorate) at the apex of the Nile Delta and *Suez* located in the Suez Governorate. Stool specimens were obtained from persons living in the city proper. The majority of these people are merchants, shop keepers, general laborers and civil servants. Sanitary conditions are variable but in most areas poor. The majority of people live under crowded conditions but are not exposed directly to irrigation systems and some of the other usual means of food and body contamination common to small farming communities. Most of the specimens from Suez were obtained from the secondary school.

Communities intermediate between true agricultural and desert dwellers. Wadi Nassim (Qena Prov.) is a small village at the margin of the desert and the narrow cultivated area along the Nile. Village people consist of laborers, animal herders and those engaged in limited farming. *Wadi Natroun* (Western Desert) is a small settlement in a desert depression half way between Cairo and Alexandria. The majority of families are employed by a local mining (salt and sodium carbonate) company. Farming in this area is restricted to small gardens because of nonfertility of soil, lack of rain and no irrigation facilities.

Desert communities. El Arish (Sinai Prov.) is a city of several thousand population on the Mediterranean coast. It is in an arid area with a minimum of agriculture and gardening; these are restricted to small areas adjacent to wells. Some of the men net fishes in the Mediterranean but the larger number are employed as laborers at the nearby military establishment. Study specimens were obtained from people on the southern or desert side of the city. *South Sinai.* Materials were obtained from Bedouins encountered on the desert or camped in the wadis along trails leading to the central and southern part of the Sinai Peninsula. One series was obtained from Bedouins in a "city" of goatskin tents at the base of Mt. Sinai.

SURVEY FINDINGS

The incidence of intestinal protozoa and helminths, based upon study of two samples (direct smear for protozoans and helminths but with emphasis on the former; AMS-III concentration method for detection of helminth eggs) from a single stool specimen from 1,408 persons living in different localities is given in Table 1. Localities from which materials were obtained are indicated on Map 1. Since the number of stools obtained from some villages was small and most lots represented peoples covering a wide range of age, no attempt has been made to present data by age groups. In Table 1 no consideration is given to the occupational status of the adults who were employed. In discussing parasitism among the different peoples studied, however, reference, when appropriate, is made to the nature of their work, certain customs, and to the general living conditions in relation to the parasite picture.

Ten species of intestinal protozoa were found, as well as the eggs and/or larvae of 19 species of helminths. A number of stools contained the eggs of a tyroglyphoid mite. Table 1 also indicates the percentage of the population samples studied in which no protozoans or helminths were detected, as well as those negative for all intestinal fauna.

I. Protozoa. The incidence of protozoans in the 13 different localities definitely point to a very generalized and effective medium or means for transmission of intestinal fauna. Although the role of flies in the transmission of intestinal protozoa in Egypt is not clearly understood, and in most studies is a point of conjecture, flies constitute one of the major health hazards in Egypt (Weir, *et al.*, 1952). Observations indicate that flies, especially of the *Musca domestica* complex (*M. domestica vicina* Macq., the predominant species) would be incriminated in parasite transfer. These flies are of considerable significance (Sabrosky, 1952) since they are characteristically domestic and are numerous in cafes, bazaars, food stores, animal sheds and, above all, in most homes. The presence of flies, sometimes in unbelievable densities, is the one factor that is common to most stations from which specimens were

TABLE 1

Incidence (per cent) of intestinal protozoa and helminths in people living in different type localities in Egypt*

	Fishing village	Urban communities			Farming & agricultural communities					Intermediate between agricultural & desert communities		Desert communities	
	El Burg	Alexandria	Cairo	Suez	Abu Rawash	Faiyum	Shirbin	Sindbis	Tanash	Wadi Nassim	Wadi Natroun	El Arish	South Sinai
Protozoa													
Entamoeba histolytica (LR† only)	7	6	4	7	9	8	7	11	9	11	10	8	10
Entamoeba histolytica (SR† only)	32	17	20	19	13	28	17	23	11	27	28	20	25
Entamoeba histolytica (LR & SR together)	17	7	13	11	17	6	2	17	11	11	14	28	21
Total *E. histolytica*	56	30	37	37	39	42	26	51	31	49	52	56	56
Entamoeba coli	73	50	58	54	74	56	46	69	57	67	62	64	76
Endolimax nana	50	37	32	34	43	39	21	33	35	60	46	64	51
Iodamoeba bütschlii	23	8	6	11	15	12	9	13	14	39	8	24	46
Dientamoeba fragilis	3	4	2	9	1	4	—	6	2	21	8	14	2
Giardia lamblia	9	4	15	20	21	5	4	25	13	4	24	18	16
Chilomastix mesnili	9	5	5	9	7	5	12	13	2	21	16	22	21
Trichomonas hominis	8	2	1	1	11	3	—	4	3	—	2	12	17
Entermonas hominis	11	13	2	9	18	10	—	17	7	4	12	22	14
Isospora hominis	—	—	—	—	1	—	2	—	—	—	—	—	—
Helminths													
Hookworm (*Ancylostoma*)	10	12	—	11	70	21	11	12	38	16	14	2	2
Ascaris lumbricoides	49	5	6	25	72	23	37	79	31	7	18	46	2
Enterobius vermicularis	—	9	3	13	9	9	9	7	6	3	14	—	—
Heterodera	2	3	4	9	9	13	7	2	4	—	—	—	—
Strongyloides stercoralis	2	—	—	3	4	—	—	1	—	—	2	—	—
Trichostrongylus	5	3	—	4	9	12	—	6	3	—	6	—	—
Trichuris trichiura	15	27	—	3	9	4	4	2	5	—	2	—	—
Diphyllobothrium	—	1	—	—	1	—	—	—	—	—	—	—	—
Hymenolepis diminuta	—	1	1	5	2	—	—	—	—	—	—	—	—
Hymenolepis nana	7	3	16	41	34	12	4	30	26	3	32	2	3
Taenia	1	2	—	2	—	1	—	1	1	—	2	—	—
Dicrocoelium dendriticum	—	1	—	—	—	—	—	—	1	—	2	—	—
Fasciola sp	—	2	—	1	5	1	4	—	—	—	—	—	—
Heterophyes heterophyes	31	1	—	1	2	1	4	1	—	—	—	—	—
Haplorchis pumilo	2	—	—	—	—	—	—	—	—	—	—	—	—
Haplorchis taichui	9	2	—	2	—	—	—	—	—	—	—	—	—
Opisthorchis	—	1	—	—	—	—	—	—	—	—	—	—	—
Schistosoma haematobium	—	1	1	4	18	4	4	11	13	—	—	—	—
Schistosoma mansoni	11	3	1	7	11	6	4	29	59	—	4	—	12
Tyroglyphoid mite	—	5	1	1	1	24	—	6	—	—	—	—	—
Negative for protozoa	5	6	5	5	7	4	9	4	14	1	—	2	2
Negative for helminths	9	7	15	11	2	13	11	5	7	63	14	42	87
Negative for both protozoa and helminths	4	7	10	10	1	9	9	1	2	11	14	8	6
Number of stools examined	96	291	173	149	100	98	46	100	122	70	50	50	63

* Given as nearest whole number.

† LR = large race *E. histolytica* with cysts measuring more than 10μ; SR = small race with cysts less than 10μ.

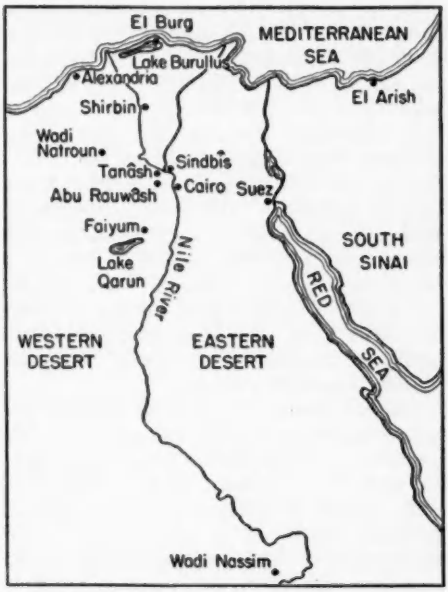
obtained. A considerable number of flies were present even in rather remote and isolated desert communities.

Entamoeba coli was the most common protozoan found among the peoples of Egypt, the maximum incidence of 76 per cent being recorded from samples taken in southern Sinai. The greatest rate of infection for *Entamoeba histolytica* was also found in these collections. Such high rates of incidence reflect emphatically the low sanitary conditions under which most of these peoples live and also serve as guides suggesting an abundance of other species of intestinal protozoa. Furthermore, it is logical to assume that contaminated hands in family food containers may contribute to parasite transmission among these Arab peoples. In most of the recent surveys (Chandler, 1954; Lawless, *et al.*, 1956; Kuntz and Lawless, 1958) conducted in Egypt the incidence for *E. coli*, as in this survey, was greater than that for the total of *E. histolytica*.

It is of interest to note that one specimen of *E. coli* had ingested several cysts of *Giardia lamblia*.

The total rates of infection for *E. histolytica* ranged from 26 to 56 per cent but did not equal the incidence which we have recorded in a previous study (Lawless, *et al.*, 1956) in Lower Egypt. Since our data are based upon examination of a single sample it seems logical to assume that the greater part of the population would have been shown to harbor *E. histolytica* had examinations been made.

The small race was predominant in all collections, the ratio of small to large race ranging from 1.5:1 to 5:1. Large and small races are listed separately since it is believed that the two races may bear a relationship to the clinical aspects of amebic parasitism. As pointed out in the works of others in Egypt, the high percentage of infection with *E. histolytica* is not consistent with the case reports of amebic dysenteries. Diarrheas are extremely common in most villages but it is probable that the majority of these result from bacterial infections (Weir, *et al.*, 1952). No attempt has been made in the present study to correlate intestinal disturbances and ailments with the presence of amebas. Possibly the low rates of true amebic dysenteries are explained in part by the fact that a fair proportion of persons reported as infected with *E. histolytica* harbor the small, nonpathogenic type. Continued recognition and separate listing of the different types of *E. histolytica* seems desirable. Hoare (1957) has recently suggested that the small race is entitled to an independent taxonomic status and Burrows (1957) has revealed that *E. hartmanni*, frequently confused with the dwarf *E. histolytica*, may be quite common. In the present study, however, the use of the MIF technic would not have permitted recognition and/or differentiation of *E. hartmanni* and the small *E. histolytica*.



MAP 1. Localities from which study specimens were obtained.

Some of the stools with only the large race *E. histolytica*, and others with both large and small races, contained mucus and erythrocytes (rbc). As intimated above, however, it is not believed that the presence of rbc was necessarily associated with the presence of pathogenic *E. histolytica* since a number of fecal samples containing only the dwarf type also contained mucus and macroscopically detectable rbc. Very few stools, even those with rbc and the large race of *E. histolytica*, contained Charcot-Leyden crystals. Eight stools contained *E. histolytica* large race, with ingested rbc. We are unable to explain why the majority of these should have been found in the villagers of Baltim.

Except for the rock-hard, dehydrated specimens obtained from Bedouins living on the open desert and accustomed to a minimal water intake, the majority of stools from which study samples were drawn were soft to mushy and a number were semi-liquid. Consequently, many of the protozoa, when present, occurred in the trophozoite as well as in the cystic stage. Prompt and effective fixation by MIF probably contributes to the fact that *Dientamoeba fragilis* was detected in all lots of stools. This ameba which frequently is entirely overlooked in survey work, was detected in 1 to 21 per cent of the fecal samples. The latter figure, slightly greater than that recorded by Chandler (1954) making a study in Sindbîs, is probably a record.

The incidence herein reported for other amebas and flagellates is rather high but consistent with previous survey figures given for Egypt (Chandler, 1954; Wells and Blagg, 1956; Lawless, *et al.*, 1956). Ranges of incidence for *Giardia lamblia* are rather broad but, as expected, the higher figures apply to fecal specimens obtained from population samples in which children predominated.

There were only two cases of infection by *Isospora hominis*. This may not necessarily represent the rarity of this coccidian, but rather the shortcomings of technics employed. Our results may well have been different had we employed the zinc sulfate flotation technic recommended for recovery of *Isospora*.

Although we have examined several thousand stools in Egypt we have failed to detect *Balantidium coli*. On one occasion prior to development of the MIF method, members (Wells and Robinson, unpublished data) of our laboratory using the standard, iron-hematoxylin stained smear for fecal samples also failed to find *B. coli* in 209 persons residing in several "pig villages" just outside Cairo. These peoples cared for pigs continually, lived in huts associated with the pig pens, pigs inhabited the same quarters as the pig keepers, and both adults and children were living under conditions which permitted repeated contamination of hands, and perhaps food, by pig excreta. Nevertheless, several of a series of smears prepared from fecal samples obtained at random from pigs contained numerous *Balantidium coli* cysts.

II. Helminths. In contrast to data obtained for the intestinal protozoa, and as indicated in Table 1, certain expected relationships were found to exist between incidence and species of helminths found, and type of locality in which the people resided. Some of the rather pronounced differences in incidence were found to be related to the occupations of families, their customs and, in general, the ecological nature of the communities from which stools were obtained. In regard to some of the species of helminths listed, there are definite relationships between life-cycles and local conditions which foster or deter parasite propagation and transmission.

The eggs and/or larvae of 19 species of helminths, including the plant nematode *Heterodera*, were encountered. Ranges of incidence were great, *Ascaris* dominating the list with 72 per cent recorded for one village. A brief discussion regarding parasitism by some of the more important or common helminths is given separately below.

Ancylostoma. The range of incidence for *Ancylostoma* was much broader than might be expected, especially for the agricultural communities. This may have been due, in part, to an influence of timing since study materials were obtained at different seasons over a period of 2 years. Furthermore, infection rates would be less accurate or representative, if, by chance, our collection of samples had coincided with periods when a good proportion of the population had been recently treated. In the case of Abu Rauwâsh, it seems likely that the infection status may have approached 100 per cent had a series of stools been examined from each individual. Observations on the site indicate that, without question, living and sanitary conditions in this village are such as to encourage hookworm propagation. The incidence of 12 per cent which we observed in Sindbîs is comparable to that given by Chandler (1954) from the same village. Such low incidences as those observed in the desert communities of El Arish and Sinai are expected since the arid conditions under which these peoples live undoubtedly destroy most helminth eggs.

We are unable to account for the absence of hookworm in 173 specimens taken in the central part of Old Cairo. It must be noted, however, that the majority of the people in this area live in crowded tenements, many two or three floors above the street. In contrast to the fellaheen, many of the city dwellers do not have the daily

contact with contaminated soil and seldom travel beyond the limits of the city to increase their chances for infection. However, it would seem that the promiscuous deposition of human feces on the ground between buildings and around the doorsteps of crowded apartments, where numerous children play, would permit some hookworm transmission.

No attempt has been made in the present study to make egg counts or estimate hookworm burdens. In 1954 Chandler, working in Sindbîs, revealed that hookworm infections are light; perhaps an average of 2 pairs per person. Apparently hookworm disease is much less of a problem than it generally is assumed to be in Egypt. Discrepancies in the figures for hookworm incidence as given by different investigators working in the same or similar agricultural communities are probably due to variations in methods of detecting helminth eggs.

Ascaris. In most surveys conducted in Egypt *Ascaris lumbricoides* has been and continues to be the most common helminth. Our data indicate general relationships between locality types and degree of parasitism. As expected, the agricultural communities show higher rates of infection since the fellaheen in these villages live in houses in which the yards and floors are highly contaminated with helminth eggs. Actually, the incidences of *Ascaris* in the cities of Cairo and Alexandria are unusually low for Egypt, suggesting, as in the case of *Ancylostoma*, that confinement within the city resulting in less contact with contaminated soil, reduces the opportunities for infection.

We are unable to explain satisfactorily the 46-per cent incidence found in the arid area of El Arish. However, questioning at the time collections were made revealed that a number of persons from whom specimens were obtained travelled some distance to work at military camps. Possibly conditions at the site of their employment were such as to allow infection. The low rate of 2 per cent was to be expected of the Bedouins of South Sinai since the majority of people live in the open where high temperatures and lack of moisture reduce *Ascaris* propagation to a minimum. A good proportion of the population at El Arish and in Sinai are semi-isolated and they seldom travel to the Nile Valley where there would be greater opportunity to contract helminth infections. Local physicians report that infections with *Ascaris* and hookworm often occur among those few who have spent time in the agricultural areas of the Nile Delta.

In contrast to the findings of Scott (1939), also working in Egypt, our data indicate that some families tend to be more heavily parasitized by *Ascaris* and *Trichuris* than other families living in the same community. Based upon general observations in Egypt we agree with Scott (1939) that the prevalence of *Ascaris* is due in great part to hand-to-mouth transfer of contaminated soil present in and around human dwellings. This is further borne out by Chandler's (1954) egg-count data on samples of soil taken in and around homes in the Sindbîs area, and by the fact that infection rates dropped as a result of improvement in village sanitation.

Eggs of ascarids other than *Ascaris lumbricoides* were observed in four samples. Two of these were identified as *Toxocara*. The closeness of the farming people to their domestic livestock, including pets, would suggest a greater degree of parasitism originating in lower mammals.

Enterobius. Since our data are based entirely upon examinations of stool specimens, the incidence listed for *E. vermicularis* is probably lower than was actually the case. In view of infection rates up to 14 per cent (Wadi Natroun) found by this method, it seems likely that the use of anal swabs would have revealed infections in excess of 50 per cent.

Trichostrongylus. This parasite was found in eight of the 13 series of collections studied. In most cases the egg densities were light and the present figures are considerably lower than those given in other recent studies (Lawless, *et al.*, 1956; Chandler, 1954) in the Nile Delta. We found only 6 per cent of the people in Sindbîs infected whereas an infection rate of 18 per cent occurred in young children of the same village examined by us earlier (Kuntz, *et al.*, 1958). The AMS-III concentration technic revealed a number of eggs in specimens which were negative by direct-smear examination.

In our opinion, based upon egg measurements, there were at least two species of *Trichostrongylus* present; one with eggs measuring 65 to 90 μ and the other 101 to 108 μ in length. Several species of *Trichostrongylus* (Belding, 1952) have been reported for Egypt. Certainly the close association of man and his domestic animals suggests the

possibility of a much heavier infestation by this group of nematodes than was found.

Trichuris. The whipworm, when present, frequently accompanied *Ascaris* but the over-all incidence of this parasite was not great. Higher infections in the coastal village of El Burg and Alexandria were to be expected since the higher humidity and added moisture characteristic of the coastal localities probably allows survival of *Trichuris* eggs. Effects of arid climatic conditions on prevalence of the whipworm are strongly in evidence in the desert and semi-desert communities where only 1 of 233 samples contained eggs.

Diphyllobothrium. The finding of *Diphyllobothrium* is of unusual interest since autochthonous cases apparently have not been reported previously. Of the three infected persons in the Alexandria series, one, a young woman, stated that she had not travelled outside Egypt or even far beyond the city limits. The other two cases may possibly have been introduced as a result of travel, or by fish products carried into Egypt from Turkey or surrounding areas where *Diphyllobothrium* is known to occur. Alexandria is a cosmopolitan city with many Greeks, Italians and Turkish peoples and the accidental introduction of infected fishes is quite possible. The fourth case, a 9-year-old girl from the village of Abu Rauwâsh near Cairo, seems to represent another autochthonous infection.

Although the possibility of infection of man by animal diphyllobothriids is not to be ruled out, the eggs detected by us resembled those of the *D. latum*.

Hymenolepis. There were eight infections with *Hymenolepis diminuta*. This rate is considerably below that reported by Chandler (1954) for the village of Sindbîs. As the latter author has pointed out, food in native habitations has an excellent chance of being contaminated by numerous rodents and arthropods which supposedly transmit species of *Hymenolepis*. However, it is obvious that, in Sindbîs, the transmission of *H. diminuta* is not very effective. Actually, the chances for parasite establishment would seem to be much greater than our data on infection indicate, since identifiable remnants of lice, grain beetles, flies, thrips, etc., were found in several fecal samples.

Eggs of the dwarf tapeworm, *Hymenolepis nana*, were observed in some fecal samples from each of the 13 localities and the rates of infection which we observed in four localities are considerably greater than in some previous reports (Chandler, 1954; Lawless, *et al.*, 1956) but do not surpass the incidence which we have recorded in the younger children of a typical Egyptian village (Kuntz and Lawless, 1958). In a number of cases the egg densities were heavy. The incidence as determined by direct-smear preparations was increased slightly after application of the AMS-III technic for study of Formalin-fixed specimens.

Taenia. Nine of the 1,408 stools contained eggs of *Taenia* (supposedly *Taenia saginata*). There is no apparent correlation between occurrence and the localities from which fecal samples were taken. A low over-all incidence was to be expected since the great laboring class of peoples in Egypt does not eat beef regularly. Our figures are not exactly consistent with the finding of the cysticerci of *T. saginata* in slaughter houses. These cysticerci are not uncommon and during several visits to the abbatoirs of Cairo we found carcasses with moderately heavy infections.

The presence of some of the trematodes listed in Table 1 is of interest since several have not heretofore been reported as parasites of man in Egypt. Our study, which was somewhat superficial, may have been even more revealing had there been opportunity to follow up and recheck those persons infected with unusual species of helminths.

Dicrocoelium and *Fasciola*. Eggs of *Dicrocoelium dendriticum* which we observed during the present study were identical with those which we had previously detected in some abundance in stool specimens from the peoples of western Turkey (Kuntz, *et al.*, 1958). These eggs appeared to be viable. However, it is difficult to determine whether these represent genuine or spurious infections. Of course, the same can be said of *Fasciola*. A total of 11 persons passed stools which contained eggs of *Fasciola* and most appeared to be viable. In our examination of numerous wild and domestic animals in Lower Egypt we found *Fasciola hepatica* and *F. gigantica* to be common. The latter was removed from most cattle and water buffalo examined.

The single stool with *Opisthorchis* eggs probably represents an accidental infection of man by a parasite of lower mammals.

Heterophyids. In our opinion the discovery of an apparent "heterophyid complex" in Lower Egypt is one of the most interesting results of the present study. Our attention was directed to this

group of trematodes prior to the beginning of the present study, when 99 per cent of approximately 4,000 snails (*Pirenella conica*) collected from El Burg produced heterophyid type cercariae. Laboratory feedings of metacercariae and examination of wild mammals and birds revealed the presence of several species of heterophyids (Martin and Kuntz, 1955; Kuntz and Chandler, 1956).

Although our data are based mainly upon a study of eggs in fecal preparations we list three species of heterophyids, i.e., *Heterophyes heterophyes* and two species of *Haplorchis*. Unfortunately, only adult *H. heterophyes* was obtained from two men in Baltim (near El Burg) subsequent to their treatment with hexylresorcinol. *Heterophyes* has been considered a parasite mainly of peoples dwelling on the shores of the brackish-water Lake Burullus, Lake Manzalla, etc. However, we detected heterophyid eggs in seven of the 13 localities studied. Wells and Blagg (1956) examined the stools of 367 children from nearby Baltim and found 36 per cent to contain *H. heterophyes*, a figure comparable to ours.

The finding of heterophyids in persons residing some distance from the brackish-water-lakes areas, i.e., Abu Rauwâsh, Sindbîs, etc., was to be expected since large quantities of fish, especially mullet (*Mugil*, a second intermediate host) are shipped to Cairo and other communities of Lower Egypt. Although most of the fish are salted, it seems likely that some are consumed before all metacercariae succumb to the salt brine. Furthermore, Wells and Randall (1956) found metacercariae of *Heterophyes* in fish from the inland brackish-water Lake Qarun in the Faiyum. We found *H. heterophyes* eggs in a stool from one person residing in this area near Lake Qarun.

A few stools also yielded heterophyid eggs similar to those of *H. heterophyes* but smaller in size, i.e., 16 to 20 μ by 9 to 10 μ. These have not been listed separately since their identity is uncertain. Some of these occurred in fecal samples with *H. heterophyes*. It may be of interest to note that Khalil (1932) while working on the life history of *H. heterophyes* in the Lake Manzalla area, described a new species (*Qasr Ainy*) and listed man as a definitive host. Khalil stated, however, that the eggs were indistinguishable from those of *H. heterophyes*.

The earlier work of Witenberg (1929) and recent examinations of numerous vertebrates in our laboratory (Wells and Randall, 1956; Kuntz and Chandler, 1956) have shown that heterophyids are common in Egypt and the Middle East. Unfortunately, information concerning the significance of this group of trematodes as parasites of man is lacking. However, autopsy reports and collections of several thousand adult parasites from individuals post mortem indicate that the peoples from Lake Burullus and adjacent areas may be heavily infected. This parasite has been of little concern medically since heavily infected persons live in somewhat isolated localities and few come to autopsy in the hospitals of Cairo and the larger cities where unusual parasitic conditions would be noted. Not to be overlooked is the fact, as noted above, that over 99 per cent of the *Pirenella conica* taken from the lake bottom in El Burg were infected with several species of heterophyids. Such an unusual phenomenon in parasitism is due in part to local customs of the inhabitants. Many people regularly wade into, and defecate directly into the lake water. In addition, the fecal containers from the homes situated just a few yards from the lake shore, are emptied into the brackish water in the area where snails were collected.

Several species of fish seined from the area of snail collection were heavily infected with the metacercarial stage of heterophyids. Wells and Randall (1956) have listed the fishes from which heterophyids were obtained by experimental feedings. It is of epidemiologic interest that another local custom also accounts for heterophyid infections. We were informed by the local peoples that the fishermen frequently eat the first fish of a new catch raw, as they begin a new fishing cruise on the lakes. Obviously such customs and a locale where nearly all peoples are concerned one way or another with the fishing industry explains the high rates of incidence.

Schistosoma. The over-all incidence of *Schistosoma haematobium* and *S. mansoni* found in this study is considerably lower than was expected of Egypt. There are certain obvious relationships between the occurrence of schistosomiasis and the locality in which studies were made. Members of the farming and agricultural communities naturally are exposed to more schistosome cercariae than the people who spend part of their time on the desert or herding animals in other arid areas. Both Sindbîs and Tanâsh are located in an endemic area and show a high incidence of *S.*

mansoni, however the rates of infection we found are lower than we have obtained previously in making spot checks. As an example, in an earlier study of fecal samples (not included in this survey) from school children of Tanâsh, 78 per cent of the stools from 6- to 12-year-old children contained mucus, macroscopic blood and eggs of *S. mansoni*. The incidence of *S. haematobium* was much greater than that given in Table 1.

The comparatively low incidence of the schistosomes found in the samples from the cities of Alexandria, Cairo and Suez is not very surprising and supports the contention given earlier that these peoples probably spend the greater part of their time within the cities and only infrequently visit agricultural areas where they could be exposed to infection. The occurrence of *S. mansoni* in the peoples of arid Sinai may seem difficult to explain. However long-standing infections by the schistosomes are common and our data cards revealed that infected persons had worked, lived or visited relatives in the Nile Valley or Delta at some time prior to this survey.

The occurrence of the tyroglyphoid mite eggs is associated with food types and this, in turn, to some extent with seasons. Certain types of food are available during different times of the year, depending upon abundance of fresh vegetables and fruits. Obviously the rather high rate of 24 per cent of stools from the people of Faiyum with these eggs suggests that cereals, local cheese, or other foods with these mites are being consumed in quantity at the time of collection of fecal specimens.

The negative data in Table 1 further substantiate the general relationships existing between locality and presence or absence of intestinal protozoa and helminths in man. In general the percentage of persons with no helminths far outnumbered those with no protozoans. The percentages of persons free of intestinal fauna seem high since in some instances, e.g., Wadi Nassim, El Arish, South Sinai, two or three species of protozoa occurred in more than 50 per cent of the samples examined. The Bedouins of the Sinai peninsula were least parasitized; 87 per cent having no helminths. Only 6 per cent, however, were totally devoid of intestinal fauna. At the other extreme is Wadi Natroun in which all 50 contributors harbored protozoans.

SUMMARY

Although our study material was represented by fecal preparations from more than 1,400 Egyptians, the number of species of protozoa found was not greater than observed in our previous studies involving far smaller samples of the population. Larger population samples, however, more strongly emphasize that the relationship between type of locality and presence of intestinal protozoans, and that between locality and prevalence of helminths, must be considered separately. Their establishment is dependent upon different means of transmission. Undoubtedly many of the protozoa are passed from person to person by contaminated hands and by food contaminated by hands and/or flies. Many of the peoples in this part of the world are unhygienic in their habits and one heavily contaminated hand thrust into a bowl which serves the entire family provides an effective vector of protozoan cysts and, in some instances, probably trophozoites. These modes of transfer are not greatly influenced by the unusually dry habitats in which many of the Egyptian peoples live. This conclusion is supported by the high incidence of intestinal protozoa in the semi-desert and desert communities.

Entamoeba coli is the most common intestinal protozoan among the Egyptian peoples and the total incidence of the large and small races of *E. histolytica* is much greater than that listed for other parts of the world. Perhaps the majority of the population would have been found harboring both species had a series of specimens from each person been examined. *Balantidium coli* was not detected in our studies and *Isospora hominis* occurred in only two persons.

This study of materials from a wide range of community types demonstrates the relationship between parasitism by some of the helminths and the habitat in which these peoples live. This was to be expected since successful establishment of infection involves intermediate hosts and favorable conditions for proper maturation of the helminth eggs outside the human body.

The present study lists 19 helminths, several species of which, (e.g., *Opisthorchis*, *Haplorchis*, *Dicrocoelium* and *Fasciola* of the Trematoda, and the cestode, *Diphyllobothrium*) are not considered parasites ordinarily found among the Egyptian peoples. *Ascaris* is the most common helminth and hookworm (*Ancylostoma*) apparently is much

less common than might have been expected for Egypt. Eggs of *Dicrocoelium* and *Fasciola* were detected in stools but do not necessarily represent true infections. A study of infections in snails, examination of vertebrate hosts in Lower Egypt, and the present survey have revealed an apparent "heterophyid trematode complex".

Our data indicate that most of the Egyptian peoples harbor some type of intestinal fauna. A number of the peoples are fairly free of helminths; the inhabitants of the arid areas of Sinai being most averminous, with 87 per cent helminth-free. The figures for individuals positive for protozoa, in general, run considerably higher than those for the helminths. *All* samples from one village (Wadi Natroun) contained protozoa.

REFERENCES

Belding, D. L., 1952. *Textbook of Clinical Parasitology*. New York, Appleton-Century-Crofts, Inc.

Burrows, R. B., 1957. *Endamoeba hartmanni*. *Amer. J. Hyg.*, **65:** 172–188.

Chandler, A. C., 1954. A comparison of helminthic and protozoan infections in two Egyptian villages two years after the installation of sanitary improvements in one of them. *Amer. J. Trop. Med. and Hyg.*, **3:** 59–73.

Hoare, C. A., 1957. Symposium on the laboratory aspects of amoebiasis. I. Introduction. *Trans. Roy. Soc. Trop. Med. and Hyg.*, **51:** 302–305.

Hunter, G. W., Hodges, E. P., Jahnes, W. G., Diamond, L. S., and Ingalls, J. W., 1948. Studies on schistosomiasis. II. Summary of further studies on methods of recovering eggs of *S. japonicum* from stools. *Bull. U. S. Army Med. Dept.*, **8:** 128–131.

Khalil, M., 1932. The life history of a heterophyid parasite in Egypt. *Compt. Rend. Cong. Internat. Med. Trop. et Hyg.*, **4:** 137–146.

Kuntz, R. E., and Chandler, A. C., 1956. Studies on Egyptian trematodes with special reference to the heterophyids of mammals. I. Adult flukes, with descriptions of *Phagicola longicollis* n. sp., *Cynodiplostomum namrui* n. sp., and a *Stephanoprora* from cats. *J. Parasitol.*, **42:** 445–459.

Kuntz, R. E., Lawless, D. K., and Langbehn, H. R., 1958. Intestinal protozoa and helminths in the peoples of western (Anatolia) Turkey. *Amer. J. Trop. Med. and Hyg.*, **7:** 298–301.

Kuntz, R. E. and Lawless, D. K., 1958. Acquisition of intestinal protozoa and helminths by young children in a typical village of Lower Egypt. *Amer. J. Trop. Med. and Hyg.*, **7:** 353–357.

Lawless, D. K., Kuntz, R. E., and Strome, C. P. A., 1956. Intestinal parasites in an Egyptian village of the Nile Valley with emphasis on the protozoa. *Amer. J. Trop. Med. and Hyg.*, **5:** 1010–1014.

Martin, W. E., and Kuntz, R. E., 1955. Some Egyptian heterophyid trematodes. *J. Parasitol.*, **41:** 374–382.

Sabrosky, C. W., 1952. House flies in Egypt. *Amer. J. Trop. Med. and Hyg.*, **1:** 333–336.

Sapero, J. J., and Lawless, D. K., 1953. The MIF stain-preservation technique for the identification of intestinal protozoa. *Amer. J. Trop. Med. and Hyg.*, **2:** 613–619.

Scott, J. A., 1939. Observations on infection with the common roundworm *Ascaris lumbricoides*, in Egypt. *Amer. J. Hyg.*, **30:** 83–116.

Weir, J. M., Wasif, I. M., Farag Rizk, J., Attia, S. M., and Abdel Kader, M., 1952. An evaluation of health and sanitation in Egyptian villages. *J. Egyptian Publ. Health Ass'n.*, **27:** 56–114.

Wells, W. H., and Blagg, W., 1956. A survey of human intestinal parasites in a fishing village of northern Egypt. *Amer. J. Trop. Med. and Hyg.*, **5:** 266–268.

Wells, W. H., and Randall, B. H., 1956. New hosts for trematodes of the genus *Heterophyes* in Egypt. *J. Parasitol.*, **42:** 287–292.

Witenberg, G., 1929. Studies on the trematode family Heterophyidae. *Ann. Trop. Med. and Parsitol.*, **23:** 131–239.

BIOLOGICAL NOTES ON *MARISA CORNUARIETIS*, A PREDATOR OF *AUSTRALORBIS GLABRATUS*, THE SNAIL INTERMEDIATE HOST OF SCHISTOSOMIASIS IN PUERTO RICO[1]

FREDERICK F. FERGUSON

Puerto Rico Field Station, U. S. Public Health Service

AND

JUAN R. PALMER

Bilharzia Control Unit, Puerto Rico Department of Health, San Juan, Puerto Rico

The striking ability of the ampullarid snail *Marisa cornuarietis* to interfere with growth of the snail intermediate host of schistosomiasis, *Australorbis glabratus*, was first observed in September, 1952, in Quebrada San Anton, near Rio Piedras, Puerto Rico (Oliver-Gonzalez, *et al.*, 1956). Subsequently, Chernin and coworkers (1956) confirmed the predatory pattern by laboratory studies showing that, because of its omnivorous and voracious nature, *Marisa* consumed more than its share of the available food and ate *Australorbis* eggs and young as well. Field studies on snails in Puerto Rican streams and ponds were started in June, 1953, and from them the following generalized biological data on *Marisa* have been gathered.

Marisa eggs are laid in smooth, whitish, oblong, gelatinous masses measuring about 2 inches long and 1 inch thick. Such masses most commonly are attached to vegetation but are also sometimes attached to concrete, rock, or wooden surfaces. Generally, eggs are deposited only in quiet, vegetated areas of a stream or impoundment, and always are placed less than 1 foot from the water surface. Plant stems are commonly found enveloped by egg masses, which are never seen floating. As the egg masses reach the hatching stage, they become brownish and ragged. Some 20 to 30 eggs are deposited in each mass.

Young *Marisa* have reddish-brown shells. Adults are mostly dark brown, with the exception of a few members of a colony which will develop three planetary, thin, chocolate-colored stripes as adults. Young and medium-sized snails move erectly on the substrate, but the largest snails move at about 15 degrees from the perpendicular. Motion is slow but steady, as they search for food. Ordinarily, *Marisa* will not show clumping tendencies, but groups of snails align themselves in rows around a common source of food.

Both *Marisa* and *Australorbis* are planorbioid in shell morphology. *Marisa* reaches a maximum diameter of 45 mm in nature. Such specimens may be 18 mm thick. They have an average of 3.75 suture whorls on the upper surface at this size. The plane of the aperture lies at an angle of 10 degrees with the axis of the spiral cavity at that point. Outer striations transversely circumscribe the body whorl, especially pronounced near the aperture. Locally, *Australorbis* rarely measures more than 30 mm in diameter, and has about 6 whorls. *Marisa* maintains a globose shape until it is about 20 mm in diameter, gradually assuming its "planorbioid" nature afterwards. The sexes cannot be differentiated upon the basis of gross external characters.

Chernin, *et al.*, (1956) found that *Marisa* readily ate *Cabomba*, *Elodea*, dwarf *Sagittaria*, carrot, cabbage, lettuce, celery, tomato, and paper towelling in the laboratory. In nature, they are often seen eating waste paper, but prefer any semirotten leaf or stem. Although they will eat fresh plant materials of many types, they do not choose grass leaves except as a last resort. Impounded populations naturally rise and fall relative to amounts of food available. The most commonly eaten plant is the elephant-ear-type growth called "malanga" (*Xanthosoma atrovirens*) which lines margins of most streams and many ponds. *Marisa* rapidly destroys stands of water lily (*Castalia ampla*) and might be useful in destroying various stands of aquatic vegetation as an adjunct to mosquito control.

For our field studies on the biological control of *Australorbis* (Ferguson, *et al.*, 1958), *Marisa*

[1] Contribution of the Communicable Disease Center, Bureau of State Services, U. S. Department of Health, Education and Welfare, and the Department of Health, Commonwealth of Puerto Rico.

have been cultured in large (20 feet x 15 feet) concrete tanks in water about 12 to 18 inches deep. Almost all individuals are confined to the bottom of these tanks. The snails thrive in masses of water hyacinth, using this both as food and as an egg-laying surface. If raised in vegetation-free water they must be fed daily, on almost any soft, succulent plant.

Marisa commonly lives in the upper water levels of streams and impoundments at depths not in excess of about 1 foot, while viable *Australorbis* may commonly be taken at depths of 4 to 5 feet in heavily populated lakes, and has been recovered once from a depth of 15 feet by an Eckman dredge. *Marisa* closely follows the leafy vegetation line in a body of water, at the submargins of soil banks. As pond waters recede on drying, the vegetation-free margins become perilous zones for *Marisa*. Such conditions are also uninviting for *Australorbis*, which must compete with the larger snail for diminishing food supplies, shelter, and egg-laying surfaces. Both snails are unable to return to a stream once they are carried onto lateral flats in storms. It is more difficult to establish *Marisa* in streams than in ponds since stream plantings may be washed out and destroyed by storms.

Survival of *Marisa* in ditches subject to periodic manual cleaning and channel rectification is poor compared to that of *Australorbis*. During excessively low-water levels when drying ponds reach a terminal state of muddiness, *Marisa* dies more rapidly than *Australorbis*. *Marisa* has not been observed to persist under damp soil-plant debris, as has *Australorbis*.

Marisa thrives in most Puerto Rican habitats of *Australorbis*. In one example, the habitat range of *Marisa* actually exceeds that of the other snail, since it can be established in rocky terminal sections of mountain streams where normal and storm-condition velocities of flow militate against *Australorbis*. The salinity range of *Marisa* is unknown, yet there is evidence that it has moved along the margins of a brackish-water lagoon from one stream, to enter and disperse in another about a mile distant. The ideal impoundment habitat is that with relatively stable levels and shores emargined with vegetation and with a fair percentage of the surface covered with leafy plants. After *Marisa* has consumed the floating material, it exists on marginal materials, including grass blades and roots.

This snail attaches with relatively great strength to vegetation or other surfaces in flowing water. It is, however, easily dislodged when tactually stimulated and sinks rapidly to the bottom. Because of this, specimens are easily collected with wire sieves. *Marisa* rarely is seen floating, except in storms. Apparently, it is not able to lift itself to the surface by the generation of internal gas bubbles.

In rapid waters, the majority of a colony are always pointed upstream. Young snails measuring less than 5 mm diameter have been seen in large numbers at the inflow of farm ponds. Older snails will move up small tributaries so far that the water only partially covers them. The movement of *Marisa* against the current is more pronounced than that of *Australorbis*. For example, *Marisa* has migrated upstream for a distance of about a mile from the Los Pena station in the Quebrada San Anton since it was first discovered there in 1952. The washing action of storms has carried it downstream and into a variety of paralotic sites as well, in this stream. *Marisa* also has been observed in small outfall streams serving farm ponds, about 0.25 mile below dams.

Unlike *Australorbis*, *Marisa* will thrive in both nonpolluted and heavily polluted waters. For example, the latter snail congregates in streams at points receiving effluent from hog sties, abattoirs, and privies. Occurrence of *Australorbis* would be limited at such sites. However, gross pollution from abattoirs slows the upstream march of *Marisa*.

This species, like *Australorbis*, may be destroyed with sodium pentachlorophenate applied by power spraying in thick vegetation using a solution containing 1,000 ppm. Limited laboratory data indicate that *Marisa* is slightly more difficult to kill with chemicals than *Australorbis*.

Apparently *Marisa* has no natural enemies in Puerto Rico, and all losses of colonies in farm ponds are attributable to a combination of drying and lack of food. No parasites are found in *Marisa* upon dissection. All attempts to infect specimens of various sizes with *S. mansoni* have failed. They are not eaten by rats, which commonly prey upon local *Australorbis*, *Helisoma*, and *Ampullaria*.

The natural spread of *Marisa* over Puerto Rico has been observed to be by storm action in streams and by moderate rheotaxis. Birds and

other animals do not seem important in the dispersion, since *Marisa* are always found in accountable habitats. *Marisa* can be transported in various containers without water for periods up to 4 hours and they have been shipped successfully via air express in damp materials.

SUMMARY

The omnivorous and voracious habits of the large ampullarid snail *Marisa cornuarietis* are the basis of its predation and competitive action against *Australorbis glabratus* in Puerto Rico. Field observations have been made on the biology of *Marisa* in comparison with that of the schistosome vectorial species.

REFERENCES

CHERNIN, E., MICHELSON, E. H., AND AUGUSTINE, D. L., 1956. Studies on the biological control of schistosome-bearing snails. I. The control of *Australorbis glabratus* populations by the snail, *Marisa cornuarietis*, under laboratory conditions. *Am. J. Trop. Med. & Hyg.*, **5**: 297–307.

FERGUSON, F. F., OLIVER-GONZALEZ, J., AND PALMER, J. R., 1958. Potential for biological control of *Australorbis glabratus* the intermediate host of Puerto Rican schistosomiasis. *Am. J. Trop. Med. & Hyg.*, **7**: 491–493.

OLIVER-GONZALEZ, J., BAUMAN, P. M., AND BENENSON, A. S., 1956. Effect of the snail *Marisa cornuarietis* on *Australorbis glabratus* in natural bodies of water in Puerto Rico. *Am. J. Trop. Med. & Hyg.*, **5**: 290–296.

A SKIN-TEST SURVEY FOR HISTOPLASMOSIS AND COCCIDIOIDOMYCOSIS IN THE MIDDLE EAST[1]

KAMAL ABOU DAOUD AND CALVIN W. SCHWABE

Department of Tropical Health, Schools of Public Health and Medicine, American University of Beirut, Beirut, Lebanon

Histoplasmosis appears to be a disease of cosmopolitan distribution although, in the Middle East, autochthonous cases have been reported thus far only from Turkey (Saglam, 1946; Akün, 1950).

Coccidioidomycosis, on the other hand, is apparently limited to the southwestern United States and to a few areas of South America. Isolated cases have been reported from Italy and the Balkans (Montessori, 1941). The large-scale air transport of personnel and equipment from the endemic oil-producing sections of the United States to the oil-producing areas of the Middle East would suggest the possibility of its introduction into this part of the world. Climatic and topographic conditions similar to those of the southwestern U. S. might favor the spread of the disease once it were introduced.

In the present survey a total of 2,030 Middle Eastern nationals have been subjected to histoplasmin or coccidioidin skin tests. Of this number, 695 of those tested with histoplasmin were males, 346 females; while 648 males and 341 females were tested with coccidioidin. The age distribution of the overall group was: 20 years or less, 324; 21–30, 827; 31–40, 404; 41–50, 250; and 51 or above, 225. Of those tested, 1,128 were resident in tuberculosis sanatoria, 462 were randomly selected patients hospitalized in the American University Hospital and the Asfourieh Mental Hospital and 440 were apparently healthy individuals.

Their countries of origin were, for the histoplasmin survey, Lebanon 612, Syria 127, Qatar 75, Saudi Arabia 69, Palestine 53, Iraq 38, Pakistan 13, Kuwait 5, Egypt 5, Iran 3, other countries (Ethiopia, Afghanistan, Turkey, Cyprus, Bahrein, Libya) 41; for the coccidioidin survey, Lebanon 539, Syria 138, Palestine 75, Qatar 72, Saudi Arabia 70, Iraq 31, Iran 18, Bahrein 6, Egypt 5, other countries 35.

None of the test group reacted positively to either of the antigens. Of a control group of 22 American staff members of the American University tested with the same antigens, 6 were strongly positive to histoplasmin and 1 to coccidioidin. Attempts to isolate *Histoplasma capsulatum* or other systemic fungi from the livers of 150 local wild mammals also have been unsuccessful (Schwabe and Schinazi, unpublished data). Though the present test population was not large, the results suggest that, if these diseases occur in Lebanon and neighboring countries, their rates of infection are probably of rather low order.

[1] Portion of a study on the epidemiology of zoonotic diseases in the Middle East supported by a grant from Pfizer International. The histoplasmin was furnished through the courtesy of Parke, Davis and Company and Eli Lilly and Company; the coccidioidin through the courtesy of Cutter Laboratories. We are grateful to Dr. Charles Nucho, Hamlin Sanatorium; Dr. Nicolas Naasan, Dahr el Bashik Sanatorium; Dr. Papken Mugrditchian, Azounieh Sanatorium; Dr. Elias Khoury, Bhannes Sanatorium; and Dr. Antranig Manougian, Asfourieh Mental Hospital, for their coperation, and to John Himo for his technical assistance.

SUMMARY

None of a total of 2,030 Middle Eastern nationals tested was positive to histoplasmin or coccidioidin.

REFERENCES

AKÜN, R. S., 1950. Histoplasmosis in a cat. *J. Am. Vet. M. A.*, **117:** 43–44.

MONTESSORI, P. P., 1941. La Granulomatosi Coccidioide in Europa. *Mycopathologica*, **3:** 131–139. (original not seen; *Rev. Med. and Vet. Mycol.*, **1:** 137).

SAGLAM, T., 1946. Histoplasmosis. *Schweiz. med. Woch.*, **76:** 1153–1156.

EXPERIMENTAL AMEBIASIS—A NEW APPROACH

R. N. CHAUDHURI, T. K. SAHA AND N. ROY

School of Tropical Medicine, Calcutta, India

Losch (1875) inoculated dogs with dysenteric stools both by mouth and by rectum and was able to produce dysentery in them. At autopsy ulcers containing amebae, similar to those inoculated, were demonstrated in the colon. Kovacs (1892) adopted the intracecal route for inducing amebic infection in cats. Carrera and Faust (1949) demonstrated the high susceptibility of the guinea pig to amebic infection by injecting trophozoites into the terminal ileum after laparotomy. They attempted to detect the progress of the infection by daily examination of freshly passed stool but the findings constituted no dependable index of the severity of infection as determined after death of the animal and consequently they abandoned the examination of feces and had to depend on the autopsy of the animals for the purpose. Rees (1955), in his excellent review on the subject, remarked on the considerable difficulty that may be experienced in ascertaining the prepatent period of rodent hosts because amebae may be multiplying in the cecum and the upper colon without being demonstrable in the fecal pellets. It is evident, therefore, that investigators have been handicapped to a great extent for want of any easy method for following the course of infection.

Svanidze (1954) described a method for studying the course of amebiasis and of the changes in the cecum in experimental infections of rabbits. There are two variants of this method, the essential part of which is the production of an artificial cecal fistula opening on to the surface of the abdomen. In this connection the following mechanical device has been found satisfactory, to study the course of amebic infection in experimental animals.

Metallic fixture. (Figure 1) This consists of a hollow and threaded, round, metal tube approximately 1 inch in total length, having a small disc fixed at one end and fitted with a cap at the other. To ensure fixation to the abdominal wall a metal washer and nut are provided. This metallic fixture is fitted into the cecum of animals by operation and a permanent communication between the lumen of the cecum and the exterior is established.

METHOD

Under ether anesthesia the abdomen of a guinea pig is opened with aseptic precautions. The cecum is brought outside. A double purse-string suture of cotton thread is passed through its sero-muscular layer; an incision is made in the area surrounded by the sutures and then the discoid end of the fixture is introduced inside the cecum. Both of the purse-string sutures are then tightened around the hollow tube. The cecum is replaced into the abdominal cavity with the other end of the tube remaining outside. The abdomen is closed by double layers of sutures, one of cotton thread for the muscle and peritoneum and the other of silk thread for the skin. The washer is applied and the nut tightened to fix the tube on to the abdominal wall in a convenient position, its outer end being closed with the cap to prevent leakage of cecal contents. No food except water is given to the animals for about 16 hours before the operation and for about 24 hours following it. The skin stitches are removed on about the 6th day. The animal is ready for use by about the 10th day following operation.

RESULTS

So far 30 guinea pigs have been subjected to this operation with a mortality of only 6 per cent. The operated animals under observation with the fixture in situ have been thriving well for 8 to 9 months. The skin and the surrounding tissues are free from any ulcer or erosion. The tube, if cleansed occasionally, maintains its patency. A guinea pig with the fixture, and a diagramatic cross section of the same, are shown in Figures 2 and 3, respectively.

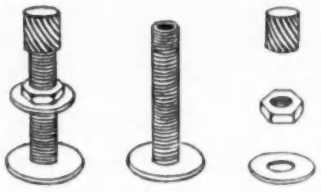


FIG. 1

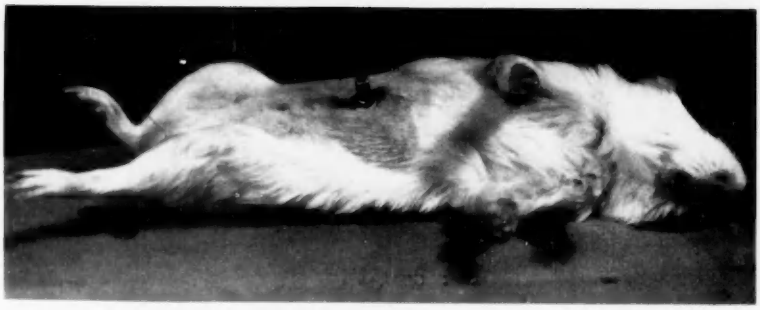


FIG. 2

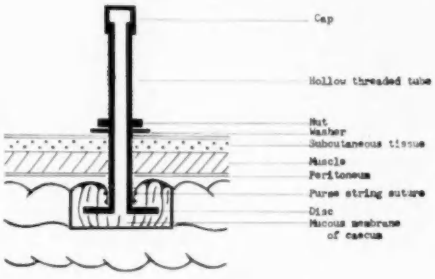


FIG. 3

COMMENT

Animals with amebic infection provide facilities for the study of the parasite under various conditions in the host. Observations thus made have had limitations. The animals usually have to be sacrificed and, in view of the fact that no two animals have the same course of infection even when kept under identical conditions, the results obtained thereby may not be comparable and reveal the exact role of the parasite in any one particular animal. Lack of a suitable method to follow the course of infection, is also a great handicap to studies on experimental chemotherapy.

The method reported herein may offer an easy means to the study of experimental amebiasis not only in detecting the onset and progress of the infection, but also in the screening and evaluations of chemotherapeutic agents. The effect of a drug may be evaluated by daily examination of cecal contents and follow-up of cured animals for relapses. The duration of treatment and the effective dose of a drug may be determined, depending on the results of cecal-content examination. If one drug fails to make an animal parasite free, others may be tried and their efficacy may be compared on the same animal. The method may also facilitate recognition of drug-resistance if any, and also study of drug-refractory strains of *E. histolytica*. Subsequent autopsy studies may reveal colonic and/or extra colonic lesions that may occur in them. Further the condition of the host may be modified by diet or other measures and their influence on the infection studied.

This simple apparatus should prove economical in experimental work as it does not involve unnecessary sacrifice of animals. Apart from an easy way of detecting parasites during the period of observation, it provides opportunities for infecting an animal intracecally repeatedly without subjecting it to abdominal operation each time.

ACKNOWLEDGMENT

This fixture was designed to facilitate studies on experimental amebiasis by the Clinical Research Unit, Indian Council of Medical Research with valuable assistance of Shri P. Ghose, laboratory assistant.

REFERENCES

CARRERA, G. M., AND FAUST, F. C., 1949. Susceptibility of the guineapig to *Endamoeba histolytica* of human origin. *Amer. J. Trop. Med.*, **29:** 647.

KOVACS, F., 1892. Beobachtungen und Versuche ueber die sogennanta amoeben Dysenterie. *Ztschr. f. Heilk.*, **13:** 509.

LOSCH, F., 1875. Massenhafte Entwickelung von Amoeben in Dickdram. *Arch. f. Path. Anat.*, **65:** 196.

REES, C. W., 1955. *Problems in amoebiasis*. Springfield, Illinois, Charles C Thomas.

SVANIDZE, D. P., 1954. New method for the study of experimental amoebiasis. *Med. Parasit and Parasitic Dis.* Moscow. No. 2, 138. (Abstract: *Trop. Dis. Bull.*, 1955, **52:** 45.)

INCIDENCE OF LEPTOSPIROSIS IN WILD MAMMALS FROM SOUTHWESTERN GEORGIA, WITH A REPORT OF NEW HOSTS FOR SIX SEROTYPES OF LEPTOSPIRES

S. McKEEVER, G. W. GORMAN, J. F. CHAPMAN, M. M. GALTON, AND D. K. POWERS

Communicable Disease Center, Bureau of State Services, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia, and Emory University Field Station, Newton, Georgia

INTRODUCTION

Investigations concerning possible wildlife reservoirs of leptospirosis were initiated by the Communicable Disease Center in 1953 at the Newton Field Station, Newton, Georgia. Since leptospires may be passed directly or indirectly from other mammals to man, the presence of any leptospires pathogenic to man in either domestic or wild mammals is a potential public health hazard. In Georgia the prevalence of leptospirosis has increased as the cattle industry has expanded. Reports by farmers in southwestern Georgia of the occurrence of the disease in herds to which no animals had been added for several years, suggest that infections in cattle may be acquired from feral reservoirs.

In Europe (Van Thiel, 1948) and Australia (Johnson, 1950) rather extensive studies have been made of wild animals as foci of leptospiral infection. Babudieri and Bianchi (1940) isolated *L. pomona* from a rat caught in rice fields during an outbreak among rice-field workers in Italy. Subsequently this serotype was isolated from a mouse (*Mus musculus lusitonicus*) in Portugal (Fraga de Azevedo *et al.*, 1951) and from a striped field mouse (*Apodemus agrarius*) in Denmark (Borg-Petersen and Fennestad, 1956). Investigations of wild animals in Australia (Doherty *et al.*, 1956) have shown the native rat (*Rattus conatus*) to be actively infected with *L. australis* A in the canefields in North Queensland. More than half of the rats caught were excreting leptospires. Serological evidence suggested that the marsupial *Isoodon obesulus* (bandicoot) can become infected and *L. autumnalis* was isolated subsequently from these animals in Malaya (Alexander *et al.*, 1955). In Czechoslovakia (Kmety, 1955) *L. australis* A has been isolated from *Apodemus flavicollis* (wood mice) and the hedgehog (*Erinaceus roumanicus*).

Although leptospirosis has been investigated extensively in domestic mammals in the United States during the past decade, only limited studies on potential wildlife reservoirs of infection have been made except on the Norway rat (*Rattus norvegicus*) (Davis, 1951; Packchanian and Sonnier, 1948). In the Western Hemisphere, *L. ballum* has been recovered from natural infections in Norway rats in Puerto Rico (Yager and Gochenour, 1952), Norway rats trapped in British Columbia (Gochenour and Yager, 1952), rural house mice (*Mus musculus*) and an opossum (*Didelphis virginiana*) trapped in Virginia (Yager *et al.*, 1953), and stock laboratory white mice (*ibid.*). Reilly (1954) has reported on the raccoon as a wildlife reservoir of *L. canicola*.

The present investigations were confined to leptospirosis in rats and mice during the first 2 years. *L. ballum* was found to occur frequently in house mice and occasionally in the roof rat (*Rattus rattus*), cotton rat (*Sigmodon hispidus*), and the old-field mouse (*Peromyscus polionotus*) (Brown, manuscript). This paper reports the results of the study of leptospirosis in marsupials, lagomorphs, large rodents, and carnivores from six counties in southwestern Georgia.

METHODS

All animals were collected with No. 2 coil-spring fox traps set at 0.1-mile intervals along farm roads. A hole slightly larger than the trap was dug, the trigger of the trap covered with a numbered cloth pad, and the trap then covered with earth level with the surrounding ground. Each trap was attached to an exposed drag.

Areas were originally selected for study on the basis of current or previous occurrence of leptospirosis in domestic animals. After leptospires were isolated from certain species, efforts were intensified to collect these species from habitats where they were most abundant irrespective of prior occurrence of leptospirosis. Likewise, con-

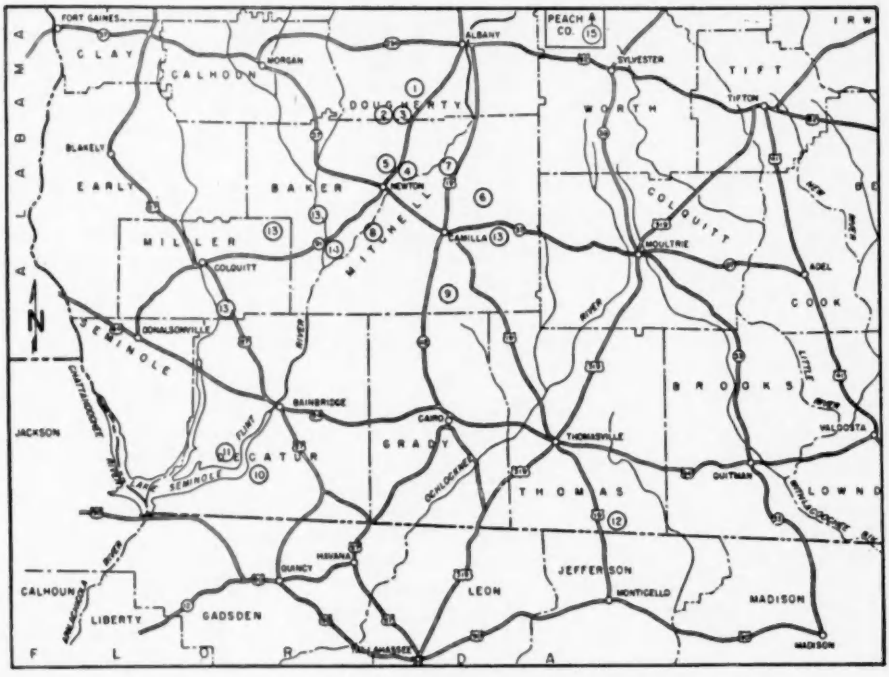


FIG. I. Map of southwestern Georgia showing locations of trapping areas

centrated collections were made from sylvan habitats where infections were found, without regard to their proximity to pastures or ranges where domestic animals were infected. General information on the type of habitat, vegetation, extent of cover, soils, and other ecological factors was obtained in the areas where trapping was done.

During exploratory studies from November 4, 1954, to September 13, 1955, data were not kept on individual traps.

From September 20, 1955, to March 20, 1956, 100 to 125 traps were set each Monday and maintained through Friday. During this period 15 areas of southwestern Georgia were sampled (Figure 1). Whenever possible, each area was trapped for 2 weeks. The habitat in which the traps were placed was recorded. All traps were baited with a mixture of fox and wildcat urine, skunk scent, and glycerine. Bait was placed at all traps on both Monday and Wednesday. Traps which caught animals or were sprung were rebaited when set. In the event of rain all traps were rebaited the following day.

Traps were checked daily, animals were removed and placed alive in numbered cages, and both the trap and cage numbers were recorded. The number of sprung traps was noted, and the number of trap nights was computed by subtracting the number of traps sprung from the total number of traps set. Percentage catch or trap success, used in this report as an expression of relative abundance, was computed for each area from the number of trap nights and number of animals caught over the entire trapping period.

At the laboratory, each animal was given a permanent number, bled by cardiac puncture, killed by injection of air into the heart, weighed, and measured. The age of the animals was determined on the basis of weight, measurement, and skull structure. One kidney or portion of one kidney was then removed aseptically and ground in a sterile mortar to which a small amount of

saline, adjusted to pH 7.2 to 7.4 with sodium phosphate ($Na_2HPO_4 \cdot 7\ H_2O$), was added. During the grinding process sufficient alundum was added to act as an abrasive and assure complete grinding. The suspension of kidney tissue was then decanted into a sterile test tube. During the early part of the study enough of the suspensions were examined and weighed to indicate that the decanted portions consisted of approximately 10 to 15 per cent suspensions of kidney tissue.

After the kidneys from an entire day's catch were ground, a portion of the suspension was removed from each test tube with a sterile Pasteur pipette, and two drops were used to inoculate 5 ml of each of four types of media in screw-cap culture tubes. The following media were used: Chang's semi-solid medium (Chang, 1947) with 10 per cent rabbit serum; Chang's semi-solid medium with 15 per cent horse plasma; Fletcher's semi-solid medium (Fletcher, 1928) with 10 per cent rabbit serum; and Fletcher's semi-solid medium with 15 per cent horse plasma. A modification of Chang's medium, as recommended by the Rocky Mountain Laboratory, National Institute of Allergy and Infectious Diseases, Hamilton, Montana, was used. Before February 17, 1955, 56 mammals were tested only with Chang's liquid and/or semi-solid medium, with horse plasma or rabbit serum added. Prior to routine use, the horse plasma and rabbit serum were determined to be free of leptospiral antibodies by routine serological procedures. After incorporation of serum or plasma into culture media, each lot was tested with leptospiral stock cultures for ability to support satisfactory growth of the organism. All media were tested for sterility before inoculation.

After inoculation, cultures were incubated for 30 days at 28°C and then examined for the presence of leptospires by dark-field microscopy. If both tubes of either base medium were contaminated, the test was not considered adequate for computing incidence of infection unless leptospires were recovered.

The serotype of isolates was determined by agglutination and cross-agglutinin-absorption tests at the Leptospirosis Research Laboratory, Laboratory Branch, Communicable Disease Center, Chamblee, Georgia.

RESULTS

Occurrence and incidence in animals. During the study 1,091 mammals, representing 14 species, were collected. Of these, 820 were tested for the presence of leptospires by culturing kidney tissue. Forty-four, (5.4%) were positive (Table 1). Sixteen of the isolates were identified as *L. ballum;* 13 as *L. pomona;* two as *L. australis* A Ballico; two as *L. grippotyphosa;* four as serotype members of the *L. mitis* Johnson-*L. hyos* (Savino and Rennella) serogroup; and two as members of the *L. hebdomadis* serogroup. Two isolates were lost prior to identification, and identification of the other three isolates has not yet been completed.

Five species of mammals were found to be hosts of one or more serotypes of leptospires. These five species accounted for 78.6 per cent of all individuals tested. The hosts were as follows: opossum, *Didelphis marsupialis* Linnaeus, for *L. ballum* and members of the *L. mitis-hyos* serogroup; gray fox, *Urocyon cinereoargenteus* (Schreber), for *L. ballum;* raccoon, *Procyon lotor* (Linnaeus), for *L. ballum, L. pomona, L. australis* A, *L. grippotyphosa,* and a member of the *L. hebdomadis* serogroup; striped skunk, *Mephitis mephitis* (Schreber), for *L. ballum* and *L. pomona;* and wildcat, *Felis rufa* Schreber, for *L. ballum* and *L. pomona.* In no instance did the infected animals show clinical evidence of leptospirosis. They did not appear to be sick, and no gross pathology of kidneys was observed.

The incidence of infection of each mammal species, by the serotype of leptospires involved, is presented in Table 1. One hundred or more specimens of four mammal species were tested. Thirteen isolates were obtained from 213 opossums, eight from 209 raccoons, 18 from 132 striped skunks, and none from 100 cottontail rabbits. Of the remaining five isolates, three were obtained from 40 wildcats and two from 52 gray foxes. The number of isolates of any one serotype obtained from the different hosts was too small to permit a valid comparison of incidence of infection by hosts and serotypes of leptospires. The highest incidence of infection was 7.6 per cent for the striped skunks infected with *L. pomona.* Five per cent of the wildcats were also infected with *L. pomona.* The incidence of infection for all other combinations of host and serotype was 3.9 per cent or less.

L. ballum was isolated from seven opossums, two gray foxes, one raccoon, five striped skunks, and one wildcat. These animals were collected on five areas in Baker, Decatur, and Mitchell Counties. Bovine leptospirosis was diagnosed

TABLE 1

Results of Tests for Leptospirae in Mammals from Southwestern Georgia November 4, 1954, to April 20, 1956

Species	Number tested	Per cent positive	Serotypes
Opossum (*Didelphis marsupialis*)	213	6.1	7 *L. ballum* 4 *L. mitis-hyos* serogroup* 2 identifications not complete
Cottontail rabbit (*Sylvilagus floridanus*)	100	0.0	
Marsh rabbit (*Sylvilagus palustris*)	2	0.0	
Gray squirrel (*Sciurus carolinensis*)	2	0.0	
Fox squirrel (*Sciurus niger*)	28	0.0	
Red fox (*Vulpes fulva*)	14	0.0	
Gray fox (*Urocyon cinereoargenteus*)	52	3.9	2 *L. ballum**
Feral dog (*Canis familiaris*)	2	0.0	
Raccoon (*Procyon lotor*)	209	3.8	1 *L. ballum** 1 *L. pomona** 2 *L. australis* A* 2 *L. grippotyphosa** 2 *L. hebdomadis* serogroup*
Striped skunk (*Mephitis mephitis*)	132	13.6	5 *L. ballum** 10 *L. pomona** 1 identification not complete 2 lost
Spotted skunk (*Spilogale putorius*)	1	0.0	
Otter (*Lutra canadensis*)	1	0.0	
Wildcat (*Felis rufa*)	40	7.5	1 *L. ballum** 2 *L. pomona**
Feral house cat (*Felis domestica*)	24	0.0	
Total (44 isolations)	820	5.4	

* Indicates new host record.

by agglutination tests on two of the areas in 1955. In 1954, and early 1955, when attempts to isolate leptospires were confined principally to small rodents in Baker and Mitchell Counties, *L. ballum* was isolated from 69 field-dwelling house mice, *Mus musculus* Linnaeus; two roof rats, *Rattus rattus* (Linnaeus); four old-field mice, *Peromyscus polionotus* (Wagner); and two cotton rats, *Sigmodon hispidus* Say and Ord (Brown, manuscript).

L. pomona was isolated from one raccoon, 10 striped skunks, and two wildcats which were collected from four areas in Baker, Decatur, Peach, and Thomas Counties. Bovine leptospirosis was diagnosed by agglutination tests on two of the areas during 1955, but there were no records of leptospirosis on the other areas. On two other areas where cows gave positive agglutination tests for leptospirosis during 1955, no *L. pomona* was isolated from wild mammals. It is of interest that *L. pomona* was not isolated from opposums.

Four isolates belonging to the *L. mitis-hyos* serogroup were obtained from opossums collected on two farms in Baker and Dougherty Counties. Serums from cows on both farms gave positive agglutination reactions for leptospirosis within the 6-month period preceding trapping activities. The identification of these isolates was reported by Galton *et al.*, (1957).

L. australis A was isolated from two raccoons collected on a forested area in Decatur County. No domestic animals except hogs, which had become wild, and dogs were present on the area. These isolates are also described by Galton *et al.*, (1957).

The following new host records were established: gray fox for *L. ballum;* raccoon for *L. ballum*, *L. pomona*, *L. australis* A, *L. grippotyphosa*, and a member of the *L. hebdomadis* serogroup; striped skunk for *L. ballum* and *L.*

pomona; wildcat for *L. ballum* and *L. pomona;* and opposum for members of the *L. mitis-hyos* serogroup.

Distribution of infections in various habitats. Leptospiral infections were found on nine of 15 areas where traps were operated (Taole 2). Incidence of infection on the positive areas ranged from 1.8 to 13.6 per cent of all animals tested. Trap success, used as an estimate of relative population density, ranged from 4.4 to 11.0. However, there was no correlation between the incidence of infection by leptospires and population density (Table 3).

Leptospires were isolated from opposums in seven areas, from striped skunks in six areas, and from raccoons in four areas. Infection rates in positive areas ranged from 3.6 to 100 per cent for opposums, from 3.4 to 100 per cent for striped skunks, and 4.3 to 20 per cent for raccoons; but there was no correlation between the incidence of infection by species of host and either mammal population density or species composition of the population.

L. ballum was found on six areas, *L. pomona* on four, *L. mitis-hyos* on two, *L. grippotyphosa* on one, *L. hebdomadis* on two, and *L. australis* A on

TABLE 2

Results of Tests for Leptospirae in Mammals from Fifteen Areas in Southwestern Georgia

Species	Area													
	1		2		3		4		5		6		7	
	Tested[a]	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
Opossum (*Didelphis marsupialis*)	11	3[1]	2		12		55	2[3]	22	1[1]	8			
Cottontail Rabbit (*Sylvilagus floridanus*)			5		4		4		6		6		7	
Marsh Rabbit (*Sylvilagus palustris*)							1							
Gray Squirrel (*Sciurus carolinensis*)													1	
Fox Squirrel (*Sciurus niger*)					1		6		2		1		3	
Red Fox (*Vulpes fulva*)			1		1		5						2	
Gray Fox (*Urocyon cinereoargenteus*)			1				1		12	1[3]	7		2	
Feral Dog (*Canis familiaris*)											2			
Raccoon (*Procyon lotor*)	4		7		5	1[2]	26		29	2[4]	7		3	
Striped Skunk (*Mephitis mephitis*)	7		3		8		2		25	6[5]	4	1[3]	5	
Spotted Skunk (*Spilogale putorius*)									1					
Otter (*Lutra canadensis*)														
Wildcat (*Felis rufa*)					3		4							
Feral House Cat (*Felis domestica*)							5		1		3		2	
Total	22	3	19	0	34	1	109	2	98	10	34	1	25	0
Per cent positive	13.6		0.0		2.9		1.8		10.2		2.9		0.0	

TABLE 2—*Continued*

Species	Area															
	8		9		10		11		12		13		14		15	
	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive
Opossum (*Didelphis marsupialis*)	7		1	1[2]	20	1[8]	38	4[10]	23		3		7	1[3]	4	
Cottontail Rabbit (*Sylvilagus floridanus*)	1		1		23		39		6				1		2	
Marsh Rabbit (*Sylvilagus palustris*)					1											
Gray Squirrel (*Sciurus carolensis*)	1															
Fox Squirrel (*Sciurus niger*)	1						2		9				1		2	
Red Fox (*Vulpes fulva*)					2		1								2	
Gray Fox (*Urocyon cinereoargenteus*)			1		14	1[8]	3		6		1				4	
Feral Dog (*Canis familiaris*)																
Raccoon (*Procyon lotor*)	5				44	3[7]	46	2[11]	27		2		4			
Striped Skunk (*Mephitis mephitis*)					38	6[8]	29	1[3]	8	2[12]			1		2	2[9]
Spotted Skunk (*Spilogale putorius*)																
Otter (*Lutra canadensis*)							1									
Wildcat (*Felis rufa*)					13	2[9]	15	1[3]	4						1	
Feral House Cat (*Felis domestica*)			1		1		4		3				3		1	
Total	15	0	4	1	156	13	178	8	86	2	6	0	16	1	18	2
Per cent positive	0.0		25.0		8.3		4.5		2.3		0.0		6.3		11.1	

* Numbers given under headings "Tested" refer to uncontaminated cultures.

1. *L. mitis-hyos* serogroup
2. *L. hebdomadis* serogroup.
3. *L. ballum*
4. *L. pomona*, 1; *L. hebdomadis* serogroup, 1
5. *L. ballum*, 1; *L. pomona*, 4; organism lost, 1.
6. Identification not complete.
7. *L. ballum*, 1; *L. australis* A, 2.
8. *L. ballum*, 2; *L. pomona*, 3; organism lost, 1.
9. *L. pomona*.
10. *L. ballum*, 3; identification not complete, 1.
11. *L. grippotyphosa*
12. *L. pomona*, 1; identification not complete, 1.

one. On the basis of the small number of isolates identified, however, there was no correlation between the incidence of infection by a given serotype of leptospires and the density of the total mammalian population or the relative abundance of the various host species. Leptospiral infections were found in every area from which more than 25 animals were tested and, as would be expected, the greater variety of serotypes was found in those areas from which large numbers of animals were collected.

The 15 areas studied may be divided into three

TABLE 3

*Relative Abundance of Fourteen Species of Mammals From Eleven Areas in Southwestern Georgia**

Species	Area										
	1	2	3	4	5	6	7	10	11	12	15
Opossum (*Didelphis marsupialis*)	2.30	1.85	1.25	3.65	2.67	1.14		0.87	1.93	1.65	2.15
Cottontail Rabbit (*Sylvilagus floridanus*)	0.26	2.78	0.58	0.73	0.64	0.80	2.34	1.66	2.46	0.43	1.61
Marsh Rabbit (*Sylvilagus palustris*)	0.13			0.18				0.04			
Gray Squirrel (*Sciurus carolinensis*)		0.31		0.18			0.20				
Fox Squirrel (*Sciurus niger*)			0.10	0.73	0.18	0.69	0.78		0.09	0.64	1.61
Red Fox (*Vulpes fulva*)		0.31	0.19			0.57	0.98	0.08	0.04		1.07
Gray Fox (*Urocyon cinereoargenteus*)	0.13	0.31	0.19	0.18	1.10	1.71	0.39	0.67			
Feral Dog (*Canis familiaris*)						0.34					
Raccoon (*Procyon lotor*)	1.15	2.78	0.49	0.55	3.31	1.37	0.98	2.06	2.56	1.65	
Striped Skunk (*Mephitis mephitis*)	1.02	0.92	0.97	0.36	2.67	0.57	1.17	1.63	1.70	0.53	1.07
Spotted Skunk (*Spilogale putorius*)					0.28						
Otter (*Lutra canadensis*)				0.18					0.04		
Wildcat (*Felis rufa*)			0.58	0.36	0.18			0.55	0.76	0.32	0.54
Feral House Cat (*Felis domestica*)				0.18	0.09	0.34	0.59	0.04	0.18	0.16	0.54
All Species	4.99	9.26	4.38	7.28	11.12	7.53	7.43	7.60	10.16	5.86	10.74

* Complete trapping information was maintained on only 11 of the 15 areas studied. For explanation see text.

groups on the basis of the predominant vegetative cover; namely, pastures and cultivated fields, pine, and pine-hardwoods. Since the interspersion of vegetative types permits all of the animals studied to range over two or more types of vegetation during the course of their daily wandering, no objective study of the vegetation was made. Classification of the areas by vegetative types is based upon subjective judgment of the senior author. Areas 1, 2, 3, 5, 7, 8, 9, and 15 were predominantly pastures and cultivated fields; areas 11 and 14 were predominantly pine, and areas 4, 6, 10, 12 and 13 were predominantly pine-hardwoods. Four locations of similar habitats, none of which yielded more than three animals, were included as area 13. A portion of all the areas except 6, 7, 9, and 15 had a cover of swamp hardwoods.

No isolations of *L. pomona* were obtained from mammals from areas which were predominantly pine, but the study of additional areas of this type may reveal the presence of the organism. There was no correlation between density of the mammalian population and incidence of infection by all serotypes with respect to the three vegetative types (Table 4), nor was the incidence of infection significantly different among mammals from areas of the three vegetative types.

Trap success for the 11 areas for which trapping records were kept, based upon the number of trap nights required to catch 1,027 animals, was 8.6 per cent. Trap success for the individual areas ranged from 4.4 to 11.0 per cent.

The following animals, which were infected as indicated, were determined to be less than 1 year old: two opposums, *L. mitis-hyos;* one

TABLE 4

Results of Trapping and Tests for Leptospirae in Mammals From Areas of Three Vegetative Types in Southwestern Georgia

Species	Pine			Pine-hardwoods			Pastures and cultivated fields		
	Caught	Tested*	Positive	Caught	Tested	Positive	Caught	Tested	Positive
Opossum (*Didelphis marsupialis*)	55	45	5	144	109	3	78	59	5
Cottontail Rabbit (*Sylvilagus floridanus*)	55	39		62	35		42	26	
Marsh Rabbit (*Sylvilagus palustris*)				2	2		1		
Gray Squirrel (*Sciurus carolinensis*)				1			3	2	
Fox Squirrel (*Sciurus niger*)	3	3		24	16		12	9	
Red Fox (*Vulpes fulva*)	1	1		13	7		12	6	
Gray Fox (*Urocyon cinereoargenteus*)	9	3		44	29	1	26	20	1
Feral Dog (*Canis familiaris*)				3	2				
Raccoon (*Procyon lotor*)	66	50	2	130	106	3	69	53	3
Striped Skunk (*Mephitis mephitis*)	39	30	1	58	52	9	58	50	8
Spotted Skunk (*Spilogale putorius*)							3		
Otter (*Lutra canadensis*)	1	1		1					
Wildcat (*Felis rufa*)	17	15	1	25	21	2	9	4	
Feral House Cat (*Felis domestica*)	7	7		12	12		6	5	
Total	253	194	9	519	391	18	319	234	17
L. ballum			6			7			3
L. pomona						6			7
L. mitis-hyos									4
L. hebdomadis									2
L. australis A						2			
L. grippotyphosa			2						
Identification not complete			1			2			
Nonviable						1			1
Per cent trap success	10.2			8.7			7.5		
Per cent positive	4.6			4.6			7.3		

* See footnote to Table 2.

striped skunk, *L. ballum;* and one raccoon, *L. australis* A. The other 40 infected individuals were determined to be more than 1 year old.

DISCUSSION

This study revealed the presence in marsupials and carnivores of six serotypes of leptospires which are potentially pathogenic to both man and domestic animals (Van Thiel, 1948; Kirschner, 1954). Any host which sheds viable leptospires may contaminate water and moist soil. Smith and Self (1955) demonstrated that leptospires could be recovered from soil 15 days after it had been infected with urine from rats previously inoculated with *L. australis* A. Domestic animals may become infected from contaminated soil or water and transmit the organism to man, or man may become infected directly from these sources. In

addition, some of the wild hosts are used for food, and all are hunted for sport and some for fur. Thus, there is a possibility that man may become infected through direct contact with these animals.

Only reference laboratories use *L. australis* A, *L. grippotyphosa*, and *L. mitis* or *L. hyos* in their batteries of antigens. Sera from hosts of *L. australis* A and the *mitis-hyos* serogroup strains showed no reaction with the antigen of *L. pomona*, the serotype used by most veterinary diagnostic laboratories. Inclusion of *L. hyos*, *L. australis* A Ballico, *L. grippotyphosa*, and *L. hebdomadis* antigens in the usual battery used by diagnostic laboratories could, therefore, lead to their detection in both man and domestic animals. In addition, more emphasis should be placed on attempts to isolate leptospires from suspected cases of leptospirosis. It is reasonable to assume that such studies might reveal the presence of these serotypes in other areas of this country.

Although *L. canicola* is known to occur frequently in dogs, especially in urban areas in Georgia, and *L. icterohaemorrhagiae* has been found in various areas in the United States in commensal rats, neither of these serotypes was found in the animals tested during the course of this study.

The limited number of isolations does not permit correlation between incidence of infection of any serotype and relative abundance of species of mammals or total mammalian population. Likewise, the data are not adequate to justify examination of correlations between prevalence and types of habitat. Studies now in progress are designed to provide additional information on these points, since feral hosts may play an important part in the maintenance and transmission of leptospires pathogenic to both man and domestic animals.

SUMMARY

From November 4, 1954, to March 20, 1956, 1,091 mammals were trapped in southwestern Georgia. Of these, 820 were tested for leptospires by culturing kidney tissue, and 44 (5.4%) were found positive. The leptospires involved were identified by agglutination and cross-agglutinin-adsorption tests as serologically homologous to *L. ballum*, *L. pomona*, *L. australis* A, *L. grippotyphosa*, and serotype members of the *L. hebdomadis* and *L. mitis-hyos* serogroups. The hosts were as follows: opossum for *L. ballum* and members of the *L. mitis-hyos* serogroup; gray fox for *L. ballum;* raccoon for *L. ballum*, *L. pomona*, *L. australis* A, *L. grippotyphosa*, and a member of the *L. hebdomadis* serogroup; striped skunk for *L. ballum* and *L. pomona;* and wildcat for *L. ballum* and *L. pomona*. No correlation was found between the incidence of infection by a given serotype of leptospires and density of the various species of mammals or the total mammalian population.

The isolation of *L. australis* A., *L. grippotyphosa*, and organisms belonging to the *L. mitis-hyos* and *L. hebdomadis* serogroups is of primary significance. Only a few reference laboratories are routinely testing for the presence of antibodies to these pathogens in either man or domestic animals. There is a need for inclusion of antigens of these serotypes of leptospires in routine agglutination tests and for more intensive efforts to isolate leptospires from suspected cases in humans and domestic animals.

The following new host records were established: gray fox for *L. ballum;* raccoon for *L. ballum*, *L. pomona*, *L. australis* A, *L. grippotyphosa*, and a member of the *L. hebdomadis* serogroup; striped skunk for *L. ballum* and *L. pomona;* wildcat for *L. ballum* and *L. pomona;* and opossum for members of the *L. mitis-hyos* serogroup.

REFERENCES

Alexander, A. D., Wetmore, P. W., Evans, L. B., Jeffries, H., and Gleiser, C. A., 1955. Classification of leptospirae isolates from Malaya, Thailand, and N. Borneo. *Am. J. Trop. Med. & Hyg.*, **4:** 492–506.

Babudieri, B., and Bianchi, L., 1940. Untersuchungen uber ein epidemisches Vorkommen der Reisfelderleptospirose in der Provinz Pavia. *Ztschr. Immunitätaforsch.*, **98:** 37–75.

Borg-Petersen, C., and Fennestad, K. L., 1956. A field rodent (*Apodemus agrarius*) as carrier of *Leptospira pomona* in Denmark. *J. Am. Vet. M. A.*, **128:** 204–205.

Chang, S. L., 1947. Culture medium for leptospiras. *J. Infect. Dis.*, **81:** 28–34.

Davis, David E., 1951. The relation between the level of population and the prevalence of leptospira, salmonella, and capillaris in Norway rats. *Ecology*, **32:** 465–468.

Doherty, R. L., Emanuel, M. L., and Moore, N., 1956. An outbreak of leptospirosis in North Queensland. *Med. Jour. Austral.*, I, **43:** 276–281.

Fletcher, W., 1928. Recent work on leptospirosis, tsutsugamushi disease, and tropical typhus in the Federated Malay States. *Trans. Roy. Soc. Trop. Med. & Hyg.*, **21:** 265–288.

FRAGA DE AZEVEDO, J., VALENTE, J. S., AND DE S. NOVOS QUEIROS, J. J., 1951. Dados sobre as Leptospiras de Portugal. *Ann. Inst. Med. Trop.*, **8:** 621. Cited by Borg-Petersen in *J. Am. Vet. M. A.* **128:** 204–205 (1956).

GALTON, MILDRED M., POWERS, D. K., MCKEEVER, S., AND GORMAN, G. W., 1957. The identification of two leptospiral serotypes hitherto not found in the United States. *Pub. Health Rep.*, **72:** 431–435.

GOCHENOUR, W. S., JR., AND YAGER, R. H., 1952. Classification of North America leptospires. *Proc. Am. Vet. M. A.*, 89th Annual Meeting, Atlantic City, pp. 178–183.

JOHNSON, D. W., 1950. The Australian leptospiroses. *Med. Jour. Austral.*, **37:** 724–732.

KIRSCHNER, L., 1954. Recent studies on leptospirosis in New Zealand. Infection with a new type (*Leptospira mitis* Johnson) (syn. *Leptospira hyos*) in man and animals. *New Zealand M. J.*, **53:** 119–128.

KMETY, EMIL, 1955. Leptospirosenherde in der [redacted] Slowakei. *Zentralb. Bakt.*, **163:** 464–476.

PACKCHANIAN, A., AND SONNIER, A. B., 1948. Incidence of leptospirosis in man and rodents in Galveston, Texas. *Rep. on Biol. and Med.*, **6:** 453–460.

REILLY, JAMES R., 1954. The raccoon as a wildlife reservoir of *Leptospira canicola*. *N. Y. Fish and Game J.*, **1:** 220.

SMITH, D. J. W., AND SELF, H. R. M., 1955. Observations on the survival of *Leptospira australis* A in soil and water. *J. Hyg.*, **53:** 436–444.

VAN THIEL, P. H., 1948. *The Leptospiroses.* x + 231 pp. Leiden, Universitaire Pers Leiden.

YAGER, ROBERT H., AND GOCHENOUR, W. S., JR., 1952. Leptospirosis in North America. *Am. J. Trop. Med. & Hyg.*, **1:** 457–461.

YAGER, R. H., GOCHENOUR, W. S., JR., ALEXANDER, A. D., AND WETMORE, P. W., 1953. Natural occurrence of *Leptospira ballum* in rural house mice and in an opossum. *Proc. Soc. Expt. Biol. & Med.*, **84:** 589–590.

ERYTHROMYCIN IN AMEBIC LIVER ABSCESS

A. J. WILMOT, S. J. POWELL AND R. ELSDON-DEW

The Amoebiasis Research Unit[1] *and the Department of Medicine, University of Natal, Durban*

There are many reports showing that erythromycin is effective in the treatment of intestinal amebiasis. In our hands it gave an immediate cure rate of 71% (Powell *et al.*, 1958). Anderson *et al.*, (1955) and Villarejos (1955) have shown that "hepatic amebiasis" or "amebic hepatitis" may respond to this drug. For this reason it seemed important to determine whether erythromycin is effective in the treatment of proved amebic liver abscess.

MATERIAL AND METHODS

Erythromycin stearate, 500 mg, was given every 6 hours for 10 days to six African male patients in whom the clinical diagnosis of amebic liver abscess had been confirmed by the aspiration of characteristic pus. In five cases *Entamoeba histolytica* was demonstrated in the pus which in all cases was bacteriologically sterile. In no patient was there clinical evidence of other disease. Diiodohydroxyquinoline was also given to enhance activity against possible bowel amebiasis. Further aspiration was done when clinically indicated. The patients were observed in hospital for a minimum period of 28 days and kept longer when symptoms demanded.

RESULTS

Two patients responded satisfactorily. After 10 days treatment one had shown no response; the abscess, which presented itself as an epigastric mass, was enlarging despite repeated aspiration. The patient was given emetine and responded well. One patient deteriorated and on the 4th day emetine was given as it was thought dangerous to continue with erythromycin alone. Two other patients appeared to respond satisfactorily to erythromycin initially, but in one symptoms had returned by the 25th day and *E. histolytica* was found in aspirated pus. The remaining patient relapsed 18 days after discharge and amebas were present in the pus.

[1] The Amoebiasis Research Unit is sponsored by The South African Council for Scientific and Industrial Research, The Natal Provincial Administration, The University of Natal, The United States Public Health Service (Grant No. E-1592).

DISCUSSION

As treatment of four cases out of six resulted in undoubted failure it was felt that further trial with erythromycin was unnecessary and unjustifiable. It is difficult to determine whether erythromycin has any activity at all in liver abscess. The only drugs of proven efficacy in this condition are emetine and chloroquine, and before the advent of these, some cases recovered on drainage alone. Therefore, the recovery of some cases of liver abscess during chemotherapy is not necessarily a test of activity of the drug. However, the failure of treatment in four cases out of six is indisputable evidence that the activity of erythromycin, if any, is slight and falls far short of that of emetine or chloroquine in which the response is excellent.

It has been our experience in the past that reports of the efficacy of a drug in "amebic hepatitis" may lead to its use in amebic liver abscess with possibly tragic results, and we think that erythromycin alone should not be used in this condition.

We have shown that chlortetracycline is ineffective in amebic liver abscess (Wilmot *et al.*, 1952). In the course of therapeutic trials with tetracycline and its derivatives in amebic dysentery, we have seen cases in which liver abscess developed, although the dysentery had responded. This has been used as an argument that tetracycline acts indirectly in amebic dysentery by altering the bacterial flora. Although its antibacterial spectrum is different from that of tetracycline, erythromycin may act in a similar manner.

SUMMARY

Erythromycin, 500 mg, was given every 6 hours for 10 days to six patients suffering from amebic liver abscess.

Two cases apparently responded, the remainder either failed to respond or relapsed.

By comparison with emetine and chloroquine, erythromycin is ineffective in amebic liver abscess.

ACKNOWLEDGMENT

We are grateful to Dr. S. Disler, Superintendent of King Edward VIII Hospital, for facilities. We wish to thank Messrs. Abbott Laboratories for supplies of erythromycin stearate.

REFERENCES

ANDERSON, H. H., NELSON, T. L., CARBONO, C., AND DIAZ, J., 1955. Erythromycin stearate against systemic amebiasis in Columbia. *Am. J. Trop. Med. & Hyg.*, **4**: 693–698.

POWELL, S. J., WILMOT, A. J., AND ELSDON-DEW, R., 1958. A comparison of erythromycin, spiramycin and novobiocin in the treatment of acute amoebic dysentery. *J. Trop. Med. & Hyg.*, **61**: 67–70.

VILLAREJOS, V. M., 1955. Treatment of amebiasis with erythromycin. *Am. J. Trop. Med. & Hyg.*, **4**: 699–704.

WILMOT, A. J., ARMSTRONG, T. G., AND ELSDON-DEW, R., 1952. Aureomycin in amebic liver abscess. *Am. J. Trop. Med. & Hyg.*, **1**: 429–434.

THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE

Combining The American Journal of Tropical Medicine
and The Journal of the National Malaria Society

VOLUME 7

EDITED BY

M. FROBISHER

EDITORIAL BOARD

G. ROBERT COATNEY, *Chairman*

PAUL C. BEAVER
WILLIAM McD. HAMMON
JOHN M. HENDERSON
HARRY MOST
LLOYD E. ROZEBOOM
ALBERT B. SABIN
LEON H. SCHMIDT

BALTIMORE, MD.
THE WILLIAMS AND WILKINS COMPANY
1958

INDEX

A

ABADIE, S. H., 329
Acanthocephalid parasite of marmosets, 90
Acquisition of intestinal protozoa and helminths by young children in a typical village of Lower Egypt, 353
ACTH and cortisone in trichinosis, 611
Acute African amebiasis: Clinical laboratory studies and response to MA-307, 201
ADAMS, E. B., 197
Aedes aegypti in southwestern Georgia, 309
Aedes polynesiensis Marks in Tahiti, 512
Africa, trypanosomiasis in, 546
African amebiasis, 201
Africans, feeding habits of anophelines amongst, 543
Allergens, tropical, 141
ALTMANN, G., 51
Amazon Valley, virus antibody survey in, 36
Ameba-bacteria relationship in amebiasis, 392
Amebiasis, acute African, 201
Amebiasis, ameba-bacteria relationship in, 392
Amebiasis, experimental, 644
Amebic liver abscess, 197
Amebic liver abscess, emetine and chloroquine in, 197
Amebic liver abscess, erythromycin in, 656
(Anatolia), helminths in the peoples of western, 298
ANDERSON, HAMILTON H., WILMOT, ALEXANDER J., FREEDMAN, LEONARD, AND ANDERSON, JEANETTE VAN D., Acute African amebiasis: Clinical laboratory studies and response to MA-307, 201
ANDERSON, JEANETTE VAN D., 201
ANDREWS, JUSTIN M., Introduction to the 22nd Craig lecturer, Dr. L. L. Williams, Jr., 257
ANDREWS, JUSTIN M., Presentation of the Joseph Augustin LePrince award for 1957, 345
ANDREWS, JUSTIN M., Tropical allergens and allergic diseases. Presidential Address, 1957, 141
Anophelines, feeding habits of, 543
Antibiotics, resistances of shigellosis to, 51
Antimalarials, 207
Arachnidism, necrotic, 165
ARNOLD, ARTHUR, 471
Arthropod-borne virus infections, (*see also specific viruses*), 323
Arthropods, insecticide resistance in, 74
ASAKURA, SOICHI, 295
Ascariasis, eosinophilic lung in, 100
Ascariasis, therapy of, 329
ASENJO, CONRADO F., RODRIQUEZ-MOLINA, R., CANCIO, MARTA, AND BERNABE, RAFAEL A., Influence of very low-fat diets with and without gluten, on the endogenous-fecal-fat excretion of patients with tropical sprue, 347
ATKINS, J. A., WINGO, C. W., SODEMAN, W. A., AND FLYNN, J. E., Necrotic arachnidism, 165
Australorbis glabratus, control of, 491
Australorbis glabratus, *Marisa cornuarietis*, a predator of, 640
Axenic cultivation of helminths, 1

B

Bacteria, amebic abscess and, 17
Bailey K. Ashford Award, 1
BALSAM, THEODORE, AND SHAFFER, JAMES G., The effect of viable bacteria on the capacity of a strain of large-race *Entamoeba histolytica* to produce abscesses in the hamster liver, 17
BAPTISTA, ANTONIO M., 84
BARTZIS, IDA LOUIS, 392
BAUMAN, PRESTON M., 603
BEAVER, PAUL C., AND DANARAJ, T. J., Pulmonary ascariasis resembling eosinophilic lung, 100
BERNABE, RAFAEL A., 347
BEVIER, GEORGE A., 25
BIAGI, F., FRANCISCO, AND TAY, JORGE, A precipitation reaction for the diagnosis of cysticercosis, 63
Biochemical investigations on DDT-resistance in the human body louse, *Pediculus humanus humanus*, 620
Biological notes on *Marisa cornuarietis*, a predator of *Australorbis glabratus*, the snail intermediate host of schistosomiasis in Puerto Rico, 640
Bionomics of *Sabethes chloropterus* Humboldt, a vector of sylvan yellow fever in Middle America, 429
Body louse, DDT-resistance in, 620
BONNET, DAVID D., AND CHAPMAN, HERALD, The larval habitats of *Aedes polynesiensis* Marks in Tahiti and methods of control, 512
BOOK REVIEWS
Acta Leidensia, ed. by the School of Tropical Medicine, Leiden, The Netherlands, 340
Annual Review of Entomology, 138
BRAY, Studies on the Exo-erythrocytic Cycle in the Genus Plasmodium, 575
CALVERLY, My Arabian Days and Nights, 576
CHEN, Soybeans for Health, Longevity, and Economy, 140
CRISP, Simulium and Onchocerciasis in the Northern Territories of the Gold Coast, 339
DARZINS, The Bacteriology of Tuberculosis, 577

DIXON AND MASSEY, Introduction to Statistical Analysis, 574
DONLE, Jahreszeit und Witterung im Seuchengeschehen. Typhus abdominalis, Paratyphus, Ruhr, Spinale Kinderlahmung, Diphtherie, Scharlach, 221
Entomology (*see Annual*)
FAUST AND RUSSELL, Craig and Faust's Clinical Parasitology, 343
FELTON, Host-Parasite Relations in Living Cells. A Symposium Sponsored by the James W. McLaughlin Fellowship Program, University of Texas—Medical Branch, Galveston, Texas, April 27, 1956, 469
FROBISHER, Fundamentals of Microbiology, 341
GREITHER AND TRITSCH, Die Geschwülste der Haut. Ihr klinisches und feingewebliches Bild, ihre Erkennung und Behandlung, 221
GRUNDY AND MACKINTOSH, The Teaching of Hygiene and Public Health in Europe: A Review of Trends in Undergraduate and Post-Graduate Education in Nineteen Countries, 342
HAGAN AND BRUNER, The Infectious Diseases of Domestic Animals, 339
Leiden (*see Acta*)
LEIMENA, Public Health in Indonesia, 340
LEWIS, Pharmacology for Medical Students in Tropical Areas, 344
MACDONALD, The Epidemiology and Control of Malaria, 577
MARTINI, Wege der Seuchen, 137
PECK, Practical Dermatology, 139
RODENWALDT AND JUSATZ, World Atlas of Epidemic Diseases, Part II, Nos. 1–4, 135, 136
RODENWALDT AND JUSATZ, World-Atlas of Epidemic Diseases, Part III, No. 4, 469
ROTH AND WILLIS, The Medical and Veterinary Importance of Cockroaches, 577
ROUECHE, The Incurable Wound, 470
SAWITZ, Medical Parasitology for Medical Students and Practicing Physicians, 138
SMITH, CONANT, BEARD, WILLET, OVERMAN, BROWN, SHARP AND POSTON, Zinsser Bacteriology, 343
THOMPSON AND KING (edited by), Biochemical Disorders in Human Disease, 341
TURNER AND HOLLANDER, Biology of the Treponematoses, 340
WORLD HEALTH ORGANIZATION, Annual Epidemiological and Vital Statistics, 1954, 342
WORLD HEALTH ORGANIZATION, International Sanitary Regulations, 470
WORLD HEALTH ORGANIZATION, World Directory of Medical Schools, 344
WILKIE, Jordan's Tropical Hygiene and Sanitation, 138
Books received, 135, 222, 344, 468, 574
BOSHELL M, JORGE, AND BEVIER, GEORGE A., Yellow fever in the lower Motagua Valley, Guatemala, 25
BOTERO, DAVID, AND GOMEZ, JOSÉ J., The first case of sparganosis in Colombia, 597
BRAGANÇA GIL, F. M., 84
BRANCATO, FRANK P., 66
BRAY, R. S., Studies on malaria in chimpanzees. VI. *Laverania falciparum*, 20
Brazil, virus antibody survey of Amazon Valley, 36
BUCKNER, ANNETTE J., 620
BUMBALO, THOMAS S., PLUMMER, LOIS J., AND WARNER, J. ROGER, The treatment of enterobiasis in children. A comparative study of piperazine (Antepar) and pyrvinium chloride (Vanquin), 212
Bunyamwera virus, Isolation of, 579
BURNETT, H. S., 600

C

C. p. quinquefasciatus, intermediate, 505
Cali, Departamento del Valle, Colombia, 4
California, Ecological studies of wild rodent plague in San Francisco Bay area of, 411
California, ecology of plague in, 112
CANCIO, MARTA, 347
CASALS, J., 561
CASALS, JORDI, 585
CAUSEY, OTTIS R., AND THEILER, MAX, Virus antibody survey on sera of residents of the Amazon Valley in Brazil, 36
CHAKRAVARTY, H. K., 95
CHAPMAN, HERALD, 512
CHAPMAN, J. F., 646
CHATTERJEE, H. N., GHOSH, S. M., CHATTERJI, B. B., CHAKRAVARTY, H. K., AND RAY, N. R., A study of prothrombin time and thrombotic phenomena in cholera, 95
CHATTERJI, B. B., 95
CHAUDHURI, R. N., SAHA, T. K., AND ROY, N., Experimental amebiasis—a new approach, 644
CHEN, P. S., 140
Chimpanzees, malaria in, 20
China, schistosomiasis *japonica* research in, 58
Chloroquine in amebic liver abscess, 197
Chloroquine and other antimalarials, on *P. vivax*, 207
Chloroquine in urine, qualitative test for, 199
Chlortetracycline hydrochloride, Elimination of *Leptospira ballum* from a colony of Swiss albino mice by, 423
Cholera, prothrombin time in, 95
Cholera, thrombotic phenomena in, 95
CHOW, C. Y., 280
CLARKE, D. H., AND CASALS, J., Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses, 561

Clark, William, David Sachs and Hugh Williams, An outbreak of infectious hepatitis on a college campus, 268
Clinical trials of compound 11925C, 416
Clyde, D. F., and Shute, G. T., Selective feeding habits of anophelines amongst Africans of different ages, 543
Coccidioidomycosis, survey for, 643
Coco, L. J., 329
Coleman, Nell, 334, 531
Colombia, sparganosis in, 597
Comparison, A, of methods for the isolation of *Toxoplasma* from suspected hosts, 531
Comparison, A, of results of hemagglutination and dye tests for toxoplasmosis in a survey of Trinidad natives, 523
Comparative, The, value of emetine and chloroquine in amebic liver abscess, 197
Compound 11925C, clinical trials of, 416
Cook, M. Katherine, The development of a pyrimethamine-resistant line of *Toxoplasma* under in vitro conditions, 400
Cortisone and ACTH in trichinosis, 611
Cortisone in filariasis, 185
Costa Rica, *Toxoplasma* antibodies in, 334
Cotton rat, immunity to the filarial worm of the, 419
Craig Lecture, 22nd, 259
Culex p. pipiens, intermediate, 505
Culex pipiens, 526
Culex pipiens fatigans, a hybridization of, 526
Culex tritaeniorhynchus, transmission of Japanese B encephalitis virus, 365
Cultivation of *Entamoeba histolytica*, 595
Cysticercosis, precipitation reaction for, 63

D

DDT-resistance in the human body louse, 620
Daily periodicity of *Schistosoma mansoni* cercariae in Puerto Rican waters, 374
Danaraj, T. J., 100
Daoud, Kamal Abou, and Schwabe, Calvin W., A skin-test survey for histoplasmosis and coccidioidomycosis in the Middle East, 643
Davey, D. G., Human and animal trypanosomiasis in Africa, 546
Davis, E., 423
Davis, Melvin R., 66
de Azevedo, J. Fraga, Carvão Gomes, F. A., F. M. Bragança Gil, F. M., Baptista, A. M., and de Magalhães, E. M., Application of radioisotopes to the study of the metabolism of fresh-water snails, (Gastropoda-Pulmonata), 84
de Magalhães, E. Mendes, 84
de Meillon, B., 579
Dengue antibodies in residents of the Society Islands, French Oceania, 403
Dengue, experimental infection of New-world monkeys with, 406
Dengue, Japanese B and mumps epidemics on Guam, 441
Dermanyssus gallinae, infestation of a human habitation by, 627
Development, The, of a pyrimethamine-resistant line of *Toxoplasma* under in vitro conditions, 400
Development of resistance to molluscicides in *Oncomelania nosophora*, 618
Diamond, Louis S., 603
Diethylcarbamazine, treatment of filariasis with, 185
Diets, influence of very low-fat, on tropical sprue, 347
Diseases, allergic, 141
Dithiazanine, Therapy of trichuriasis and ascariasis with, 329
Dithiazanine in the treatment of helminthiasis, 600
Dolkart, Ralph E., and Halpern, Bernard, A new monophasic medium for the cultivation of *Entamoeba histolytica*, 595
Djakarta, Filiariasis in, 280

E

Earle, Alexander M., and Mellon, Jr., W. Larimer, Tetanus Neonatorum; a report of thirty two cases, 315
Ecological studies of wild rodent plague in the San Francisco Bay area of California. II. Efficiency of bacterial culture compared to animal inoculation as methods for detecting *Pasteurella pestis* in wild rodent fleas, 411
Edgren, Donald C., Palladino, Vincent S., and Arnold, Arthur, Japanese B and mumps encephalitis. A clinico-pathological report of simultaneous outbreaks on the Island of Guam, 471
Effect, The, of cortisone and prednisone on bullous reactions following treatment of filariasis with diethylcarbamazine, 185
Effect of cortisone and prednisone in filariasis, 185
Egypt, Lower, 353
Egypt, protozoa and helminths in the peoples of, 630
Elimination of *Leptospira ballum* from a colony of Swiss albino mice by use of chlortetracycline hydrochloride, 423
Elsdon-Dew, R., 656
Emetine in amebic liver abscess, 197
Encephalitis epidemics on Guam, 441
Encephalitis, Japanese B, 471, 481
Encephalitis, mumps, 471, 481
Entamoeba histolytica and bacteria, 17
Entamoeba histolytica, incidence in Cali, Colombia, 4

Entamoeba histolytica in kittens, 158
Entamoeba histolytica, medium for the cultivation of, 595
Entamoeba histolytica, Studies on the growth requirements of, 302
Enterobiasis in children, the treatment of, 212
Eosinophilic lung, in ascariasis, 100
Epidemiologic studies of concurrent "virgin" epidemics of Japanese B encephalitis and of mumps on Guam, 1947–1948, with subsequent observations including dengue, through 1957, 441
Epidemiological field kit, The, 205
Erythromycin in amebic liver abscess, 656
Eshkoll-Bruck, A., 51
Evaluation, An, of the skin scarification technique for the recovery of microfilariae of *Onchocerca volvulus*, 554
Experimental amebiasis—a new approach, 644
Experimental infection of New-world monkeys with dengue and yellow fever viruses, 406
Experimental studies on *Entamoeba histolytica* in kittens, 158
Eye, infections in Punjab, 42
Eyles, Don E., 531

F

Factors influencing transmission of Japanese B encephalitis virus by a colonized strain of *Culex tritaeniorhynchus* Giles, from infected pigs and chicks to susceptible pigs and birds, 365
Faust, Ernest Carroll, Parasitologic surveys in Cali, Departamento del Valle, Colombia. I. Incidence and morphologic characteristics of strains of *Entamoeba histolytica*, 4
Fecal sedimentation concentration procedure, 150
Ferguson, Frederick F., Oliver-Gonzalez, José, and Palmer, Juan R., Potential for biological control of *Australorbis glabratus*, the intermediate host of Puerto Rican schistosomiasis, 491
Ferguson, Frederick F., and Palmer, J. R., Biological notes on *Marisa cornuarietis*, a predator of *Australorbis glabratus*, the snail intermediate host of schistosomiasis in Puerto Rico, 640
Filarial worm of the cotton rat, immunity to the, 419
Filarial worms of cotton rats, immunity against, 70
Filariasis in Djakarta, Indonesia, 280
Filariasis, treatment of, with diethylcarbamazine, 185
First, The, case of sparganosis in Colombia, 597
Fleas, *Pasteurella pestis* in, 317
Fleas on rats, 519
Flynn, J. E., 165
Formalin-ether procedure, 150
Fox, Richard M., Man-biting mosquitoes in coastal Liberia, 215
Franz, Karl N., 558
Freedman, Leonard, 201
French Oceania, Dengue antibodies in residents of the Society Islands, 403
Frick, Lyman P., 150, 295
Frye, W. W., 329

G

Galindo, Pedro, Bionomics of *Sabethes chloropterus* Humboldt, a vector of sylvan yellow fever in Middle America, 429
Galton, M. M., 646
Georgia, *Aedes aegypti* in southwestern, 309
Georgia, incidence of leptospirosis in wild mammals, 646
Ghosh, S. M., 95
Gibson, Colvin L., 531
Gibson, Colvin L., and Coleman, Nell, The prevalence of *Toxoplasma* antibodies in Guatemala and Costa Rica, 334
Gomes, Carvão, F. A., 84
Gomez, José J., 597
Gorman, G. W., 646
Gray, H. F., 138
Gresser, I., Hardy, J. L., Hu, S. M. K., and Scherer, W. F., Factors influencing transmission of Japanese B encephalitis virus by a colonized strain of *Culex tritaeniorhynchus* Giles, from infected pigs and chicks to susceptible pigs and birds, 365
Grimes, E. F., 423
Guam, 471, 481
Guam, Japanese B, mumps and dengue epidemics on, 441
Guatemala, *Toxoplasma* antibodies in, 334
Guatemala, yellow fever in, 25
Gulati, Prem Vir, 42

H

Halpern, Bernard, 595
Hammon, W. McD., Schrack, Jr., W. D., and Sather, G. E., Serological survey for arthropod-borne virus infections in the Philippines, 323
Hammon, W., McD., Tigertt, W. D., and Sather, G. E., Epidemiologic studies of concurrent "virgin" epidemics of Japanese B encephalitis and of mumps on Guam, 1947–1948, with subsubsequent observations including dengue, through 1957, 441
Hardy, J. L., 365
Harinarain, Joseph, 42
Haskins, Willard T., A simple qualitative test for chloroquine in urine, 199

HEADLEE, W. H., 139
Helminthiasis, dithiazanine in, 600
Helminths, axenic cultivation of, 1
Helminths in the peoples of western (Anatolia) Turkey, 298
Helminths, and protozoa, acquisition of, in Lower Egypt, 353
Helminths and protozoa in the peoples of Egypt, 630
Hemagglutination-inhibition, techniques for, 561
Hemagglutination tests for toxoplasmosis, 523
Hepatitis, infectious, 268
HERMAN, CARLTON M., 603
Histopathology, The, of experimental infections in mice with *Schistosomatium douthitti*, 285
Histoplasmosis, survey for, 643
HSÜ, H. F., AND LI HSÜ, S. Y., On the size and shape of the eggs of the geographic strains of *Schistosoma japonicum*, 125
HU, S. M. K., 365
Human and animal trypanosomiasis in Africa, 546
Hybridization, A, of *Culex pipiens fatigans* Wiedemann from the Philippine Islands with American strains of the *Culex pipiens* group (Diptera: Culicidae), 526
Hystrichopsylla linsdalei, the plague-vector, efficiency of, 317

I

Immunity to filarial worms, 70
Immunity to the filarial worm of the cotton rat, 419
Incidence of leptospirosis in wild mammals from southwestern Georgia, with a report of new hosts for six serotypes of leptospires, 646
Indonesia, Filariasis in Djakarta, 280
Infectivity to mosquitoes of *Plasmodium vivax* following treatment with chloroquine and other antimalarials, 207
Infestation, An, of a human habitation by *Dermanyssus gallinae* (Degeer, 1778) (Acarina: Dermanyssidae) in New York City resulting in sanguisugent attacks upon the occupants, 627
Influence of very low-fat diets with and without gluten, on the endogenous-fecal-fat excretion of patients with tropical sprue, 347
Insecticide resistance in arthropods, 74
Intestinal protozoa and helminths in the peoples of Egypt living in different type localities, 630
Intestinal protozoa and helminths in the peoples of western (Anatolia) Turkey, 298
Introduction to the 22nd Craig lecturer, Dr. L. L. Williams, Jr., 257
Isolation of Bunyamwera virus from a naturally infected human being and further isolations from *Aedes* (*Banksinella*) *circumluteolus* Theo, 579
Isolation, The, and identification of *Trypanosoma cruzi* from raccoons in Maryland, 603
Isolation, The, and identification of Turlock virus in tissue culture, 536
Isolation and identification of western equine encephalomyelitis virus from mosquitoes by tissue culture methods, 187

J

JACOBS, LEON, 523
Japanese B encephalitis, 441, 471, 481
Japanese B encephalitis virus, Factors influencing transmission of, 365
Japanese B and mumps encephalitis. A clinicopathological report of simultaneous outbreaks on the Island of Guam, 471
JEFFERY, G. M., Infectivity to mosquitoes of *Plasmodium vivax* following treatment with chloroquine and other antimalarials, 207
JOE, LIE KIAN, CHOW, C. Y., WINOTO, R. M. P., SOEGIARTO, AND RUSAD MOH, Filariasis in Djarkarta, Indonesia, 280
JONES, FRANCES E., EYLES, DON E., COLEMAN, NELL, AND GIBSON, COLVIN L., A comparison of methods for the isolation of *Toxoplasma* from suspected hosts, 531
JOSEPHINE, SISTER M. ANN, Experimental studies on *Entamoeba histolytica* in kittens, 158

K

KAGAN, IRVING G., AND MERANZE, DAVID R., The histopathology of experimental infections in mice with *Schistosomatium douthitti*, 285
KARTMAN, LEO, MILES, VIRGIL I., AND PRINCE, FRANK M., Ecological studies of wild rodent plague in the San Francisco Bay Area of California. I. Introduction, 112
KARTMAN, LEO, PRINCE, FRANK M., AND QUAN, STUART F., Studies on *Pasteurella pestis* in fleas: VII. The plague-vector efficiency of *Hystrichopsylla linsdalei* compared with *Xenopsylla cheopis* under experimental conditions, 317
KENNEDY, B. M., 140
KEY, IRIS D., 302
KHALIL, H. M., 416
Kittens, *Entamoeba histolytica* in, 158
KOKERNOT, R. H., SMITHBURN, K. C., DE MEILLON, B., AND PATERSON, H. E., Isolation of Bunyamwera virus from a naturally infected human being and further isolations from *Aedes* (*Banksinella*) *circumluteolus* Theo, 579
KUNTZ, R. E., LAWLESS, D. K., LANGBEHN, H. R., AND MALAKATIS, G. M., Intestinal protozoa and helminths in the peoples of Egypt living in different type localities, 630
KUNTZ, ROBERT E., AND LAWLESS, DEANER K., Acquisition of intestinal protozoa and hel-

minths by young children in a typical village of Lower Egypt, 353
KUNTZ, ROBERT E., LAWLESS, DEANER K., AND LANGBEHN, H. R., Intestinal protozoa and helminths in the peoples of western (Anatolia) Turkey, 298
KURLAND, LEONARD T., 481

L

Laboratory screening of compounds for molluscicidal activity against *Oncomelania nosophora* with an immersion test and a modified plate test, 295
LAIGRET, JACQUES, 185
LAMPERT, R., 329
LANGBEHN, H. R., 298, 630
Larval, The, habitats of *Aedes polynesiensis* Marks in Tahiti and methods of control, 512
Laverania falciparum in chimpanzees, 20
LAWLESS, DEANER K. 298, 353, 630
LEE, ROBERT D., AND PARMELEE, WARREN E., Thelaziasis in man, 427
LEMON, F. R., 600
LENNETTE, EDWIN H. 187, 536
LePrince, Joseph Augustin, Award, 345
Leptospira ballum, Elimination of, from a colony of Swiss albino mice, 423
Leptospirosis in wild mammals, incidence of, 646
LI HSÜ, S. Y., 125
Liberia, man-biting mosquitoes in coastal, 215
Liberia, onchocerciasis in, 558
LIN, SUNG SHEN, 494
Liver abscess, amebic, 197
LORD, RICHARD A., Studies on the use of cortisone and ACTH in trichinosis, 611
LUNDE, MILFORD S., AND JACOBS, LEON, A comparison of results of hemagglutination and dye tests for toxoplasmosis in a survey of Trinidad natives, 523
Lung, eosinophilic, in ascariasis, 100

M

MA-307, response to, 201
MCARTHUR, JOHN, A new concept in microscope design for tropical medicine, 382
MACDONALD, E. M., AND SCOTT, J. A., The persistence of acquired immunity to the filarial worm of the cotton rat, 419
MACDONALD, ETTA MAE, 70
MCKEEVER, S., GORMAN, G. W., CHAPMAN, J. F., GALTON, M. M., AND POWERS, D. K., Incidence of leptospirosis in wild mammals from southwestern Georgia, with a report of new hosts for six serotypes of leptospires, 646
MCMILLAN, HARLAN L., Study of a naturally occurring population intermediate between *Culex p. pipiens* and *C. p. quinquefasciatus*, 505
MALAKATIS, G. M., 630
Malaria in chimpanzees, 20
Malaria eradication—Growth of the concept and its application. (22nd Annual Craig Lecture), 259
MALDONADO, JOSÉ F., AND OLIVER-GONZALES, JOSÉ, The prevalence of *Schistosoma mansoni* in certain localities of Puerto Rico: A three-year study, 386
Man-biting mosquitoes in coastal Liberia, 215
MAO, C. P., Research on schistosomiasis *japonica* in China, 58
MARBERG, K., ALTMANN, G., AND ESHKOLL-BRUCK, A., Observations on resistance to sulfadiazine and antibiotics in shigellosis, 51
MARCH, HARRY N., AND LAIGRET, JACQUES, The effect of cortisone and prednisone on bullous reactions following treatment of filariasis with diethylcarbamazine, 185
Marisa cornuarietis, a predator of *Australorbis glabratus*, 640
Marmosets, 90
MARTINI, E., 137
Maryland, *Trypanosoma cruzi* from, 603
MELLON, JR., W. LARIMER, 315
MERANZE, DAVID R., 285
Mexican farm laborers, helminthiasis in, 600
MEYER, K. F., 138
Mice with *Schistosomatium douthitti*, 285
Microfilariae, recovery of, 554
Microscope design, new concept in, 382
Middle America, Bionomics of *Sabethes chloropterus* Humboldt in, 429
Middle East, histoplasmosis and coccidioidomycosis in, 643
MILES, VIRGIL I., 112
MILLER, J. H., 329
MILLER, MAX J., An evaluation of the skin scarification technique for the recovery of microfilariae of *Onchocerca volvulus*, 554
MILLER, MAX J. AND FRANZ, KARL N., Some clinical aspects of onchocerciasis in Liberia, 558
mites (see *Dermanyssus*)
MOHR, CARL O., Relation of mean number of fleas to prevalence of infestation on rats, 519
Molluscicidal activity, laboratory screening of compounds for, 295
Molluscicides, resistance to in *Oncomelania nosophora*, 618
Monkeys, acanthocephalid parasite of, 90
Monkeys, New-world, 406
MOON, ARTHUR P., FRICK, LYMAN P., AND ASAKURA, SOICHI, Laboratory screening of compounds for molluscicidal activity against *Oncomelania nosophora* with an immersion test and a modified plate test, 295

Mosquitoes, man-biting in coastal Liberia, 215
Mosquitoes, western equine encephalomyelitis virus from 187
Motagua Valley, Guatemala, yellow fever in, 25
Mumps encephalitis, 471, 481
Mumps and Japanese B encephalitis epidemics on Guam, 441

N

Nagaty, H. F., Rifaat, M. A., Salem, S. and Khalil, H. M., Clinical trials of compound 11925C, 416
Necrotic arachnidism, 165
Neff, Beverly Jean 187, 536
New, A, concept in microscope design for tropical medicine, 382
New, A, monophasic medium for the cultivation of *Entamoeba histolytica*, 595

O

Oliver-Gonzalez, José, 386, 491
Olsen, Leroy J., 70
Onchocerca volvulus, skin scarification technique for, 554
Onchocerciasis in Liberia, 558
Oncomelania nosophora, 295
Oncomelania nosophora, resistance to molluscicides in, 618
Outbreak, An, of infectious hepatitis on a college campus, 268

P

Palitz, L. L., 139
Palladino, Vincent S., 471
Palmer, Juan R., 491
Parasitologic surveys in Cali, Colombia, 4
Parmelee, Warren E., 427
Pasteurella pestis in fleas: VII, 317
Pasteurella pestis in wild rodent fleas, 411
Paterson, H. E., 579
Peck, S. M., 139
Pediculus humanus humanus, DDT-resistance in, 620
Perry, Albert S., and Buckner, Annette J., Biochemical investigations on DDT-resistance in the human body louse, *Pediculus humanus humanus*, 620
Persistence, The, of acquired immunity to the filarial worm of the cotton rat, 419
Phage absorbed on paper disks in the rapid identification of *Vibrio comma*, 312
Philippines, arthropod-borne virus infections in the, 323
Phillips, Bruce P., Wolfe, Patricia A., and Bartzis, Ida Louis, Studies on the amebabacteria relationship in amebiasis. II. Some concepts on the etiology of the disease, 392
Phthalofyne in trichuriasis, 66
Plague, ecological studies of, 112
Plague in the San Francisco Bay area of California, 411
Pieper, Jr., Samuel J. L., and Kurland, Leonard T., Sequelae of Japanese B and mumps encephalitis. Recent follow-up of patients affected in 1947–1948 epidemic on Guam, 481
Piperazine (Antepar) and pyrvinium chloride (Vanquin), a comparative study of, 212
Plasmodium vivax, infectivity to mosquitoes of, 207
Plummer, Louis J., 212
Potential for biological control of *Australorbis glabratus*, the intermediate host of Puerto Rican schistosomiasis, 491
Powell, S. J., 197, 656
Powers, D. K., 646
Prednisone in filariasis, 185
Presentation of the Joseph Augustin LePrince award for 1957, 345
Presidential Address, 1957, 141
Prevalence, The, of *Schistosoma mansoni* in certain localities of Puerto Rico: A three-year study, 386
Prevalence, The of *Toxoplasma* antibodies in Guatemala and Costa Rica, 334
Price, Donald L., The epidemiological field kit, 205
Prince, Frank M. 112, 317
Prothrombin time in cholera, 95
Protozoa and helminths, Acquisition of intestinal, in Lower Egypt, 353
Protozoa and helminths in the peoples of Egypt, 630
Pyrvinium chloride (Vanquin) and piperazine (Antepar), a comparative study of, 212
Puerto Rican schistosomiasis, 491
Puerto Rican waters, Daily periodicity of *Schistosoma mansoni* cercariae, 374
Puerto Rico, biological notes on *Marisa cornuarietis*, 640
Puerto Rico, the prevalence of *Schistosoma mansoni* in certain localities, 386
Puerto Rico, schistosomiasis in, 640
Punjab Village, eye infections in, 42
Pyrimethamine-resistant line of *Toxoplasma*, 400

Q

Quan, S. F., Ecological studies of wild rodent plague in the San Francisco Bay area of California. II. Efficiency of bacterial culture compared to animal inoculation as methods for detecting *Pasteurella pestis* in wild rodent fleas, 411
Quan, Stuart F., 317
Quarterman, K. D., and Schoof, H. F., The status of insecticide resistance in arthropods of public health importance in 1956, 74

R

Radioisotopes in study of snails, 84
Raccoons in Maryland, *Trypanosoma cruzi* from, 603
Rats, cotton, immunity to filarial worms, 70
Rats, fleas on, 519
RAY, N. R., 95
REEVES, W. C., 138
Relation of mean number of fleas to prevalence of infestation on rats, 519
Resistance of arthropods to insecticides, 74
Resistance of shigellosis to antibiotics and sulfadiazine, 51
RIFAAT, M. A., 416
RITCHIE, LAWRENCE S., 150
RODRIQUEZ-MOLINA, R., 347
ROSEN, LEON, Dengue antibodies in residents of the Society Islands, French Oceania, 403
ROSEN, LEON, Experimental infection of New-world monkeys with dengue and yellow fever viruses, 406
ROWAN, WILLIAM B., Daily periodicity of *Schistosoma mansoni* cercariae in Puerto Rican waters, 374
ROY, N., 644
ROZEBOOM, LLOYD E., A hybridization of *Culex pipiens fatigans* Wiedemann from the Philippine Islands with American strains of the *Culex pipiens* group (Diptera: Culicidae), 526
RUBIN, MELVIN L., Studies on Toxoplasmosis, 358
RUSAD, MOH., 280

S

Sabethes chloropterus, Bionomics of, 429
SACHS, DAVID, 268
SADUN, E. H., LIN, SUNG SHEN, AND WILLIAMS, J. E., Studies on the host parasite relationships to *Schistosoma japonicum*. I. The effect of single graded infections and the route of migration of schistosomula, 494
SADUN, ELVIO H., AND WALTON, BRYCE C., Studies on the host parasite relationships to *Schistosoma japonicum*. II. Quantitative changes in the concentration of serum proteins in humans and rabbits, 500
SAHA, T. K., 644
SALEM, S., 416
San Francisco Bay, Ecological studies of wild rodent plague, 411
San Francisco Bay Area, ecology of plague in, 112
SAPPENFIELD, R. W., 329
SATHER, G. E., 323, 441
SAWITZ, W. G., 138
SCHERER, W. F., 365
Schistosoma japonicum, geographic strains of, 125
Schistosoma japonicum. I., host parasite relationships to, 494
Schistosoma japonicum. II., host parasite relationships to, 500
Schistosoma mansoni cercariae, Daily periodicity of, in Puerto Rican waters, 374
Schistosoma mansoni, the prevalance of, in certain localities of Puerto Rico, 386
Schistosomatium douthitti, infections in mice, 285
Schistosomiasis *japonica*, research on, in China, 58
Schistosomiasis, Puerto Rican, 491
Schistosomiasis in Puerto Rico, 640
Schistosomula, migration of, 494
SCHOOF, H. F., 74
SCHRACK, JR., W. D., 323
SCHULER, ROGER W., 302
SCHWABE, CALVIN W., 643
SCOTT, J. A., 419
SCOTT, J. ALLEN, MACDONALD, ETTA MAE, AND OLSEN, LEROY J., Attempts to produce immunity against the filarial worms of cotton rats by transfer of developing worms, 70
Selective feeding habits of anophelines amongst Africans of different ages, 543
Sequelae of Japanese B and mumps encephalitis. Recent follow-up of patients affected in 1947–1948 epidemic on Guam, 481
Serological, The, reactions in yellow fever, 585
Serological survey for arthropod-borne virus infections in the Philippines, 323
Serum proteins in humans and rabbits, 500
Shaffer-Frye medium, 302
SHAFFER, JAMES G., 17
SHAFFER, JAMES G., SCHULER, ROGER W., AND KEY, IRIS D., Studies on the growth requirements of *Entamoeba histolytica*. The ingestion of altered bacterial structures by *E. histolytica* in the Shaffer-Frye medium, 302
Shigellosis, resistance of, to sulfadiazine and antibiotics, 51
SHUTE, G. T., 543
Simple, A, qualitative test for chloroquine in urine, 199
Skin scarification technique for recovery of microfilariae, 554
Skin-test, A, survey for histoplasmosis and coccidioidomycosis in the Middle East, 643
SMITH, W. W., AND LOVE, G. J., Winter and spring survival of *Aedes aegypti* in southwestern Georgia, 309
SMITHBURN, K. C., 579
Snails, application of radioisotopes, 84
Society Islands, Dengue antibodies in residents of the, 403
SODEMAN, W. A., 165
SOEGIARTO, 280
Some clinical aspects of onchocerciasis in Liberia, 558
Sparganosis in Colombia, 597

Sprue, tropical, 347
Statistical evaluation of the formalin-ether (406th MGL) fecal sedimentation concentration procedure, 150
Stoenner, H. G., Grimes, E. F., Thrailkill, F. B., and Davis, E., Elimination of *Leptospira ballum* from a colony of Swiss albino mice by use of chlortetracycline hydrochloride, 423
Studies on the ameba-bacteria relationship in Amebiasis. II. Some concepts on the etiology of the disease, 392
Studies on the growth requirements of *Entamoeba histolytica*. The ingestion of altered bacterial structures by *E. histolytica* in the Shaffer-Frye medium, 302
Studies on the host parasite relationships to *Schistosoma japonicum*. I. The effect of single graded infections and the route of migration of schistosomula, 494
Studies on the host parasite relationships of *Schistosoma japonicum*. II. Quantitative changes in the concentration of serum proteins in humans and rabbits, 500
Studies on *Pasteurella pestis* in fleas: VII. The plague-vector efficiency of *Hystrichopsylla linsdalei* compared with *Xenopsylla cheopis* under experimental conditions, 317
Studies on toxoplasmosis, 358
Studies on the use of cortisone and ACTH in trichinosis, 611
Study of a naturally occurring population intermediate between *Culex p. pipiens* and *C. p. quinquefasciatus*, 505
Sulfadiazine, resistance of shigellosis to, 51
Swartzwelder, J. C., Lampert, R., Miller, J. H., Sappenfield, R. W., Frye, W. W., Abadie, S. H., and Coco, L. J., Therapy of trichuriasis and ascariasis with dithiazanine, 329

T

Takos, Michael J., and Thomas, L. J., The pathology and pathogenesis of fatal infections due to an acanthocephalid parasite of marmoset monkeys, 90
Tahiti, *Aedes polynesiensis* in, 512
Tay, Jorge, 63
Taylor, Carl E., Prem Vir Gulati and Joseph Harinarain, Eye infections in a Punjab village, 42
Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses, 561
Telfer, James G., Davis, Melvin R., and Brancato, Frank P., Effects of phthalofyne in the treatment of human *Trichuris trichiura* infection, 66
Tetanus Neonatorum; a report of thirty two cases, 315
Theiler, Max, 36
Theiler, Max, and Casals, Jordi, The serological reactions in yellow fever, 585
Thelaziasis in man, 427
Therapy of trichuriasis and ascariasis with dithiazanine, 329
Thomas, Lyell J., 90
Thrailkill, F. B., 423
Thrombotic phenomena in cholera, 95
Tigertt, W. D., 441
Tissue culture methods, 187
Toxoplasma antibodies in Guatemala and Costa Rica, 334
Toxoplasma, development of a pyrimethamine-resistant line, 400
Toxoplasma, isolation of, 531
Toxoplasmosis, dye tests for, in Trinidad, 523
Toxoplasmosis, Studies on, 358
Treatment, The, of enterobiasis in children. A comparative study of piperazine (Antepar) and pyrvinium chloride (Vanquin), 212
Trichinosis, cortisone and ACTH in, 611
Trichuriasis, therapy of, 329
Trichuris trichiura infection, phthalofyne in, 66
Trinidad natives, toxoplasmosis in, 523
Tropical allergens, 141
Tropical allergens and allergic diseases. Presidential Address, 1957, 141
Tropical sprue, 347
Trypanosoma cruzi from raccoons, identification, 603
Trypanosomiasis in Africa, 546
Turkey, helminths in the peoples of western (Anatolia), 298
Turlock virus, isolation and identification of, 536

U

Urine, chloroquine in, 199
Use, The, of dithiazanine in the treatment of helminthiasis in Mexican farm laborers, 600

V

Vibrio comma, phage in the rapid identification of, 312
Virus antibody survey in Amazon Valley, 36
Virus, Turlock, 536
Viruses, arthropod-borne (*see specific viruses*)

W

Wagner, E. D., Lemon, F. R., and Burnett, H. S., The use of dithiazanine in the treatment of helminthiasis in Mexican farm laborers, 600
Walton, Bryce C., 500
Walton, Bryce C., Bauman, Preston M., Diamond, Louis S., and Herman, Carlton

M., The isolation and identification of *Trypanosoma cruzi* from raccoons in Maryland, 603
WALTON, BRYCE C., WINN, M. M., AND WILLIAMS, J. E., Development of resistance to molluscicides in *Oncomelania nosophora*, 618
WARNER, J. ROGER, 212
WATANABE, YOSHIKAZU, Phage absorbed on paper disks in the rapid identification of *Vibrio comma*, 312
WEINSTEIN, PAUL P., Some projected uses for the axenic cultivation of helminths. (Address on receiving the Bailey K. Ashford Award), 1
WELSH, HARTWELL H., NEFF, BEVERLY JEAN, AND LENNETTE, EDWIN H., The isolation and identification of Turlock virus in tissue culture, 536
WELSH, HARTWELL H., NEFF, BEVERLY JEAN, AND LENNETTE, EDWIN H., Isolation and identification of western equine encephalomyelitis virus from mosquitoes by tissue culture methods, 187
Western equine encephalomyelitis virus, isolation and identification from mosquitoes, 187
WILLIAMS, HUGH, 268
WILLIAMS, J. E., 494, 618
WILLIAMS, JR., LOUIS L., Malaria eradication—Growth of the concept and its application. (22nd Annual Craig Lecture), 259
WILLIAMS, ROGER W., An infestation of a human habitation by *Dermanyssus gallinae* (Degeer, 1778) (Acarina: Dermanyssidae) in New York City resulting in sanguisugent attacks upon the occupants, 627
WILMOT, A. J., POWELL, S. J., AND ADAMS, E. B., The comparative value of emetine and chloroquine in amebic liver abscess, 197
WILMOT, A. J., POWELL, S. J., AND ELSDON-DEW, R., Erythromycin in amebic liver abscess, 656
WILMOT, ALEXANDER J., 201
WINGO, C. W., 165
WINN, M. M., 618
WINOTO, R. M. P., 280
Winter and spring survival of *Aedes aegypti* in southwestern Georgia, 309
WOLFE, PATRICIA A., 392
WYKOFF, DALE E., FRICK, LYMAN P., AND RITCHIE, LAWRENCE, S., Statistical evaluation of the formalin-ether (406th MGL) fecal sedimentation concentration procedure, 150

X

Xenopsylla cheopis, the plague-vector efficiency of, 317

Y

Yellow fever, Experimental infection of New-world monkeys with dengue and, 406
Yellow fever in Guatemala, 25
Yellow fever, serological reactions in, 585
Yellow fever, sylvan, 429

NOTICE TO CONTRIBUTORS

The Journal welcomes papers which contribute to the purpose of the American Society of Tropical Medicine and Hygiene. This is the advancement of knowledge of tropical and subtropical medicine and hygiene through investigation, prevention, treatment and control of diseases and deficiencies. It draws upon medicine, biology, public health, nursing, engineering and allied fields of research and application.

The American Journal of Tropical Medicine and Hygiene combines the American Journal of Tropical Medicine and the Journal of the National Malaria Society and therefore has a special concern with the investigation, therapy and control of malaria in all parts of the world, as well as a broad interest in general parasitology and arthropod-borne diseases.

Papers are ordinarily published in the order of reception, which will entail a certain delay in their appearance because of the contractual limitation on the number of journal pages available in any year. Accepted articles which raise no question of scientific priority but for which immediate publication seems important will be inserted in the next available issue of the journal if the author or sponsoring institution will pay the cost, estimate of which may be obtained from the editor. The insertion of such articles does not affect publication of other manuscripts awaiting their turn, since the inserted articles constitute additional pages in the volume without additional cost to the subscriber.

Publication will be facilitated if the following suggestions are followed:

1. *Manuscripts* should be typewritten in *English* using *double spacing* on *one side* only of 8½ x 11 inch paper and submitted *in duplicate*. Double-spacing should be used throughout the manuscript including the list of references, footnotes, and quotations.

2. The author should supply, on a separate sheet, a short title not over 50 letters for use as a running head. Also on separate sheets type (a) the authors' names and affiliations; (b) tabular matter; (c) case histories; (d) quotations; (e) formulas and other subsidiary matter in the text; (f) footnotes; (g) references; (h) legends for illustrations; (i) displayed formulas occupying more than 1 line of type, even if using the same size type as the main text.

3. Legends should be separate and not attached to, or written on, the illustration copy.

4. Positions for tables and figures in the text may be indicated on the margin of the text pages.

5. All papers should end with a brief summary.

6. *Tables* should be typed on separate sheets and be serially numbered in Arabic numerals.

7. *Figures* and *photographs* should be protected with cardboard to avoid creasing in the mails. Only glossy prints can be used. Line drawings and graphs should be made with India ink and submitted on white (not cross-section) paper. Colored illustrations can be reproduced only at the expense of the author.

8. *References* should be cited in text by author and date, and the list should be in alphabetical order according to authorship, with the date following the author's name, e.g.:

REED, L. J., AND MUENCH, H., 1938. A simple method of estimating fifty per cent endpoints, *Am. J. Hyg.* 27: 493–497.

9. *Proprietary names* of drugs or chemicals registered by commercial pharmaceutical firms may not be used in the text if there is a generic name available in the U. S. Pharmacopoeia, New and Nonofficial Remedies of the A. M. A., or the latest editions of standard medical dictionaries. In certain cases where the generic name is relatively unknown, the registered name may follow it in parentheses in the title, at the first citation in the text, and in the conclusions.

Estimating costs of reprints. Where the cost of reprints must be known in advance of publication in order to secure prior approval for their purchase, the Editor will be glad to furnish an estimate of the number of pages which the article will occupy in the Journal.

DIFCO

Antisera and Antigens

Enteropathogenic Coli Klebsiella

Salmonella Shigella

Brucella Leptospira Streptococci

★

C Protein Antiserum and Standard

★

Infectious Mononucleosis Antigens and Standards

Potent

Stable

Specific

Descriptive literature available on request

Specify

DIFCO LABORATORY PRODUCTS

BIOLOGICS CULTURE MEDIA REAGENTS